Research Article

Asian Jour Hosp Phar, Vol: 4, Issue: 3, 2024, 7-11

Asian Journal of Hospital Pharmacy

Content Available at www.ajhponline.com ISSN: 2583-0724

DETECTION OF HBV DNA BY DIRECT PCR PROTOCOL AND NESTED PCR PROTOCOL AND COMPARING THEM IN CHRONIC PATIENTS AND HEALTHY CARRIERS OF HEPATITIS B VIRUS.

Mushtaq Talib Al-Safi

Ministry of Education, General Directorate of Education in Thi-Qar, Iraq

Received: 15 June 2024 Revised: 05 July 2024 Accepted: 11 Aug 2024

Abstract

Hepatitis B virus is a major public health problem, and the outcome of infection depends on the effectiveness of the virus's interaction with the host, and in particular on the strength of the natural and conditioned response of humoral and cellular immunity. This study was conducted to assess the clinical status, genetic immunity and viral (viral) traits of Iraqi patients with chronic hepatitis B virus and healthy carriers of hepatitis B surface antigen, as well as to show the relationship between the immune status and the chronic condition of the disease. The study included (60) patients with chronic hepatitis B virus type (B) and (60) healthy hepatitis B surface antigen carriers who attended the Teaching Hospital for Gastrointestinal and Liver Diseases in Nasiriya City Al-Hussein Teaching Hospital, Thalassemia Center and the Central Blood Bank in Nasiriya Citysouthern of Iraq. for the period from the first period. From February 2022 until the end of February 2022, the average age of patients infected with chronic hepatitis B virus was 64.5 years, and the average age of healthy carriers of hepatitis B surface antigen was 35. 1 year, and the ratio of males to females was (12.2:) for chronic patients and (13.6:) for carriers of the disease. As for the deoxyribonucleic acid, it was (100%) in chronic patients and healthy carriers using the Nested type polymerase chain reaction, and at a rate of (30.0%) and (46.7%) in chronic patients and healthy carriers, respectively using the polymerase chain reaction technique. direct with a significant difference between the two interactions. The current results confirm that a nested PCR protocol to detect HBV DNA is a more sensitive way of detecting HBV in Iraq CHB patients and carriers. Recent advanced techniques such as nucleic acid assays, quantitative assays for viral load and RT-PCR for Iraqi viral hepatitis laboratories are recommended.

Keywords: Hepatitis B virus,PCR protocol,HBV DNA, Nested PCR protocol.

***Corresponding Author**

Mushtaq Talib Al-Safi

DOI: https://doi.org/10.38022/ajhp.v4i3.90

Introduction

The term (Hepatitis) is applied to a broad category of clinical and pathologic conditions, characterized histologically by hepatocellular necrosis and inflammatory cell infiltration of the liver [1] . Hepatitis results from hepatocyte damage produced by the action of varingform of chemical agents including drugs, toxins, alchohol or some pathogens such as, viruses and others [2].

However, virus still represents the most common and important causative agent of hepatitis caused by at least five different viruses which are ecologically, immunologically, and epidemiologically distinct [3]. Viral hepatitis types are: A (formerly called infectious hepatitis), B (serum hepatitis), C (formerly called post-transfusion non-A non-B hepatitis), D (Delta hepatitis), E (Enterically transmitted). F and G, cryptogenic (caused by a virus as yet unidentified) [4].

In clinical practice, chronic hepatitis is suspected in patients with elevated liver enzymes for longer than six months, however many inflammatory diseases other than chronic hepatitis produce same clinical features [5] .More than 400 million people worldwide are chronically infected by HBV.which is a very longterm health problem and at least 10% of chronically infected individuals develop cirrhosis and five years survival rate is only 55% . The virus is responsible for more than 300000 cases of liver cancer every year in the world [6].

The chronic hepatitis B virus infection are also health problems in Iraq. The reported prevalence of hepatitis B rate is (2–4) % among normal Iraqi population which is among the countries of intermediate hepatitis B endemicity[7]. Countries with intermediate or high

endemicity must have mass immunization for all infants at birth and for all risk groups [8].

The polymerase chain reaction (PCR) is an in vitro technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region [9]. PCR amplification of DNA is achieved by using oligonucleotide primers. These are short, single – stranded DNA molecules which are complementary to the end of a defined sequence of DNA template, the primers are extended on single stranded denaturated DNA (template) by a DNA polymerase, in the presence of deoxynucleoside triphosphate (dNTPs) under suitable reaction condition. This results in the synthesis of new DNA strands complementary to the template strands [10].

These strands exit at this stage as double stranded DNA molecules. Strand synthesis can be repeated by heat denaturation of the double – stranded DNA, annealing of primers by cooling the mixture and primer extension by DNA polymerase at a temperature suitable for the enzyme reaction [11]. Moreover, the diagnostic technique is not influenced by the antiviral therapy administered to the patient, so it is possible to detect the viral DNA also during the first days of treatment with a specific antiviral therapy [12].

HBV PCR can be used for the identification of low level HBV viremia in HBsAg positive subjects with active liver disease without HBV-DNA positivity in standard hybridization technique. It is also useful for the appraisal of HBV infection in liver transplantation, evaluation of antiviral treatment and for the diagnosis of HBsAg in acute or chronic liver disease [13].Conventional hybridization procedures have now been used widely for the detection of HBV-DNA in serum, tissues, and mononuclear blood cells [14]. HBV-DNA is the most direct and sensitive test for viral multiplication and semi quantitative tests have been developed, based on liquid hybridization [15]. There is a need for more sensitive tests to identify the HBV-DNA sequences, based on the following: (a) hepatitis B surface antigen (HBsAg) positive blood donors or mothers who have been shown to transmit HBV infection despite being positive for antibodies to hepatitis B e antigen (anti-HBe) and HBV-DNA negative in serum [16]. (b) some patients with HBsAg positive /anti-HBe positive chronic active hepatitis having active liver disease despite serum HBV-DNA negativity and absence of hepatitis delta virus or hepatitis C virus (HCV) coinfections, autoimmune liver disease, or other causes of liver disease ; (c) it is important to have a precise follow up of patients under antiviral treatment, to evaluate partial or complete responses and the subsequent risk of reactivation ; (d) PCR is necessary for subsequent sequencing of the amplified products [17]. (e) HBsAg negative patients with acute and chronic liver diseases have been shown to contain HBV-DNA sequences in the blood, liver, or mononuclear cells. HBV-DNA has also been shown in blood donors with no HBV serological marker [18]. Several studies have highlighted potential use of PCR for HBV-DNA detection [14].

Aims of the study: -

The present study aimed to fulfill the following goals: -

To find out a relationship between chronicity of disease (pathogenicity of disease) and certain HLA class I alleles, in addition to determine a correlation between carrier state of disease and certain HLA class I alleles.Detection of HBV DNA by direct PCR protocol and Nested PCR protocol and comparing them. in chronic patients and healthy carriers Hepatitis B virus. Also, detection of HBV by polymerase chain reaction (PCR).

Materials & methods

Immunogenic test: -

Determining HLA Class I antigen

. Nucleic acid test (NAT) according to the Groves *et al.*

Detection of the HBV DNA by PCR technique.Huet al.

Detection of HBV DNA by PCR techniques

AB analitica kit for detection of HBV DNA based on the amplification of the core region.

A. Test principle

Three nucleic acid segments are involved in the reaction: double stranded DNA template to be amplified (target DNA) and two single-stranded oligonucleotides " primers "that are designed in order to anneal specifically to the template DNA.

The DNA polymerase begins the synthesis process at the region marked by the primers and synthesizes new double stranded DNA molecules, identical to the original double stranded target DNA region, by facilitating the binding and joining of the complementary nucleotides that are free in solution (dNTPs). After several cycles, one can get millions of DNA molecules which correspond to the target sequence [19].

Note: - the sequence of primer was used 5'-TGTGACGACTGAGGTAGAAG-3' provided by AB Analitica, Italy, kit for detection of HBV DNA

The strength of an association between disease and genetic marker is generally expressed in terms of a relative risk value (RR), which indicates how many times more frequently a disease develops in individuals carrying the marker in individuals lacking it. The RR is defined by the following formula:

$$RR=\frac{a\times d}{b\times c}$$

a: Number of patients positive for the marker.

b: Number of patients negative for the marker.

c: Number of controls positive for the marker.

d: Number of controls negative for the marker.

The RR value can range from less than (negative association) to more than one (positive association). In the letter case an etiological fraction (EF) was given, which indicates how much of a disease is “due to” the disease associated factor. The EF is defined by the following formula:

$$\left(\frac{RR-1}{RR}\right)\times\left(\frac{a}{a+b}\right)$$ EF=

In the former case, a preventive fraction (PF) is given, which indicates how much of a disease is prevented by the disease associated marker. The PF is defined by the following formula:

$$PF=\frac{(1-RR)\times\left(\frac{a}{a+b}\right)}{RR\left(1-\frac{a}{a+b}\right)+\left(\frac{a}{a+b}\right)}$$

Both the EF and PF value can vary between zero (no association) and one (maximum association).

The level of significance (probability) is calculated by fisher's exact probability (p) through constructing 2X2 contingency tables from the previous four entries (a, b, c and d).to avoid a chance occurrence of an association (due to many comparisons), the P is multiplied by the number antigens tested at each HLA locus, therefore the corrected probability (Pc) is given[17].

Results

Nucleic acid Test (NAT).

In the present study, the establishment of the molecular techniques for detection of HBV DNA in chronic patients and carrier group give rise a clear picture especially when used the Nested PCR. Recent data indicates a highly significant importance (P<0.001) that is noticed between PCR direct protocol and Nested PCR protocol, there are the positivity of HBV DNA which is (30 %) in chronic group and (46.7%) in carrier group by using PCR direct protocol whereas, HBV DNA was (100%) in both group by using Nested PCR protocol, as given away in table (1) and figures (1,2).

Table: -1 the difference between chronic group and carrier group according to different PCR protocols.

Nucleic Acid Test		cases		Total
		healthy carrier HBV	chronic HBV	
PCR direct amplification of HBV DNA	Positive Count	13	10	23
	% within case	56.52%	43.48%	100 %
	Negative	15	22	37
	% within case	40.54%	59.46%	100 %
Total	Account	28	32	60
	% within case	46.67%	53.33%	100 %
Nested PCR of HBV	Positive Count	15	22	37
	% within case	40.54%	59.46%	100 %
Total	Count	13	10	23
	% within case	56.52%	43.48%	100 %

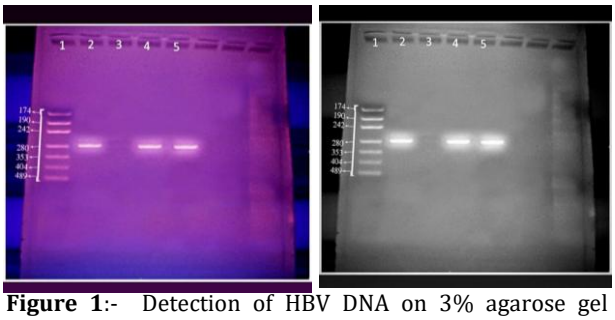

Figure 1:- Detection of HBV DNA on 3% agarose gel electrophoresis
(direct amplification) for 45 min at 100 volt. 1) DNA marker. 2) HBV positive control. 3) Negative control 4&5) HBV positive sample (270 bp band)

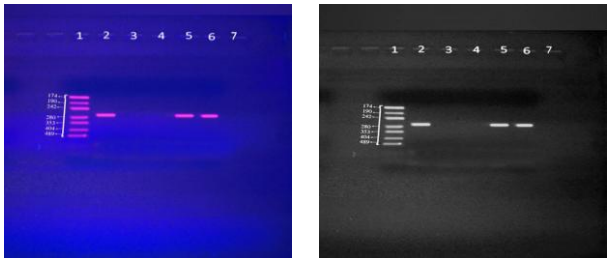

Figure 2: Detection of HBV DNA on 3% agarose gel electrophoresis
(nested amplification) for 45 min at 100 volt.
1) DNA marker. 2) HBV positive control. 3) Negative control of the direct amplification. 4) Negative control of nested amplification
5&6) HBV positive sample (258 bpband).

Discussion

Chronic hepatitis B infection is a common disease wide world, 5% of the world's population are chronic carriers [20]. also this disease is believed to be common in Iraq as well as in many other developing countries [21].

This investigation covers a wide range of parameters including virological ,immunogenetics and molecular that may have a possible relationship with infection and investigation was done on 50 patients with CHB infection and 50 individuals with HBV carrier.

In fact, age at infection seemed to be the most influencing factor in prognosis, the results of this study indicated that the mean age for chronic patients was 45.06y and for

carrier group was 35.1y. These results coincide with the previous studies done in Iraq as Kumar *et al.,* who establish that 45y was the mean age for CHB patients and Al-Fraiji, and Al-Shammary, reported that the mean age was 38 y for carrier group, also on other hand Aghdaie*et al.,* in (2022) registered that the mean age for carrier and chronic groups was 37y and 42.6y respectively.

The mean age group affected of CHB patients was higher than the mean age of carrier group which might be due to early exposure to HBV [22].egarding both groups, the males are more than females with the ratio of (2.2:1) among chronic group and (3.6:1) among carrier group. The sex differences among both groups could be explained on the basis that males may have a greater chance to come in contact with risk factors of HBV than females, or alcohol intake being common in males, which may enhance the liver damage caused by HBV infection [23].

After all, hepatitis B infection is considered to be one of the important causes of chronic liver disease all over the world. The possible routes of transmission of HBV is a multifactorial process, some of them are still controversial. The polymerase chain reaction (PCR) is an in vitro technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region [24]. In recent times, the PCR is used for detecting HBV DNA in liver and serum. The PCR has permitted to distinguish the extremely small quantities of nucleic acid for HBV in serum of patient. It is also useful for the appraisal of HBV infection in liver transplantation, assessment of antiviral treatment and for the identification of HBsAg in acute or chronic liver disease, so the detection of hepatitis B virus DNA is a reliable evidence of the presence the viral agent and its replication [22].

The establishment of the molecular techniques for identified HBV DNA in chronic patients and carrier group give away an obvious image especially when uses the Nested PCR. The current results confirm that a nested PCR protocol is more sensitive manner to detect HBV DNA than PCR direct protocol. The positivity of HBV DNA in carrier group and chronic group reached to (46.7 % and 30.0 %) respectively by employing directprotocol whereas, HBV DNA is (100%) in both groups by Nested protocol were used.

Studies by other researchers showed that HBV DNA among healthy HBV carriers and CHB patients were detected in 44.0% and 37.0% respectively of the samples using a direct amplification of HBV DNA with detection limit of 8.600 copies/mL [18].Besides that, Pawlotsky ,(2008) had detected HBV DNA by employ commercially available PCR assay with precentage of positivity was (56.0 %) among CHB patients and (48.0 %) among carrier HBV group with limit of HBV detection of 6.340 copies/mL.

On the contrary, Barlet*etal.*HBV DNA was discovered in the greatest level of CHB samples and the highest level of carrier HBV samples using a nested procedure with low HBV DNA (560copies/mL).BV samples by nested protocol with low level of HBV DNA (560copies/mL), behind that Ezea, *etal.*found the positivity of HBV DNA in chronic patients and a symptomatic HBV carrier by using nested polymerase protocol was (89% and 92%) respectively with low detection limit of HBV DNA was noticed (400 copies/mL), as well other investigator showed that a non-significant difference was noticed between a chronic patients and carrier subject as regard to nested PCR protocol [25-27].

In the present study, the comparison of the two molecular methods of HBV DNA identification revealed that the second molecular PCR was able to detect very low amount of HBV DNA in serum of patients, therefore this method is regarded as the most efficient technique.

As a final point, the current results confirmed the importance of this molecular test which leads to increase the chance of diagnosis and decrease the development of disease to end-stage of liver disease and hepatocellular carcinoma, therefore the high sensitivity and specificity of PCR permits virus detection soon after infection and even before the onset of disease, as well as early detection may give physicians a significant lead in treatment.

Conclusion

The development of molecular tools for the detection of HBV DNA in chronic patients and the carrier group has created an evident image, particularly when the Nested PCR technique is employed. As a consequence of the current findings, it is confirmed that a nested PCR protocol is a more sensitive method of detection of HBV DNA than a PCR direct technique.

References

1. Aghdaie, S. F. A., Ansari, A., &Telgerdi, J. Analysing the impact of gamification dynamics and e-WOM on impulsive buying. International Journal of Procurement Management.2022, 15(1), 40-61.
2. Al-Fraiji, S. S., & Al-Shammary, D. Survey for Electroencephalography EEG Signal Classification Approaches. In Mobile Computing and Sustainable Informatics (pp. 199-214). Springer, Singapore, 2022.
3. Ali, H. M., Al-Shuwaikh, A. M., &Manuti, K. Molecular and phylogenetic detection of Torque Teno Virus (TTV) among hemodialysis patients: A single center study. Iraqi JMS.20(1): 11-25. doi: 10.22578. IJMS. 2022, 20(3).
4. Arankalle, V. A. 14 HEPATITISVIRUSES: VIROLOGY EPIDEMIOLOGY. Tropical Hepato-Gastroenterology-E-Book. 2008 , 261.
5. Arankalle, V. A. 14 HEPATITISVIRUSES: VIROLOGY AND. Tropical Hepato-Gastroenterology. 2010, 261.
6. Girish, C., & Pradhan, S. C. Drug development for liver diseases: focus on picroliv, ellagic acid and curcumin. *Fundamental & clinical pharmacology*. 2008,*22*(6), 623-632.
7. Groves, J. A., Y. Dodd, R., Zhu, X., Linnen, J. M., &Stramer, S. L. Background rate of low-level HCV RNA in anti-HCV confirmed-positive and minipool nucleic

acid test-nonreactive blood donations. Transfusion. 2022, 62(1), 143-150.

8. Hong, J. M., Gibbons, M., Bashir, A., Wu, D., Shao, S., Cutts, Z., ...&Pawlosky, A.ProtSeq: Toward high-throughput, single-molecule protein sequencing via amino acid conversion into DNA barcodes. Iscience. 2022, 25(1), 103586.
9. Horvat, R. T., & Taylor, R. Hepatitis B and D viruses. Manual of clinical microbiology. 2015,1841-1858.
10. Hu, F., Liu, Y., Zhao, S., Zhang, Z., Li, X., Peng, N., & Jiang, Z. A one-pot CRISPR/Cas13a-based contamination-free biosensor for low-cost and rapid nucleic acid diagnostics. Biosensors and Bioelectronics. 2022, 113994.
11. Pacin-Ruiz, B., Cortese, M. F., Tabernero, D., Sopena, S., Gregori, J., García-García, S., ...& Rodríguez-Frías, F. Inspecting the Ribozyme Region of Hepatitis Delta Virus Genotype 1: Conservation and Variability. Viruses. 2022, 14(2), 215.
12. Pollicino, T., Amaddeo, G., Restuccia, A., Raffa, G., Alibrandi, A., Cutroneo, G., ... & Raimondo, G. Impact of hepatitis B virus (HBV) preS/S genomic variability on HBV surface antigen and HBV DNA serum levels. Hepatology. 2012, 56(2), 434-443.
13. Velikova, G., Booth, L., Smith, A. B., Brown, P. M., Lynch, P., Brown, J. M., & Selby, P. J. Measuring quality of life in routine oncology practice improves communication and patient well-being: a randomized controlled trial. Journal of Clinical Oncology. 2004, 22(4), 714-724.
14. Webb, G. W., Kelly, S., & Dalton, H. R. Hepatitis A and Hepatitis E: Clinical and epidemiological features, diagnosis, treatment, and prevention. Clinical Microbiology Newsletter. 2020, 42(21), 171-179.
15. Zahn, K. E., Jensen, R. B., Wood, R. D., &Doublié, S. Human DNA polymerase θ harbors DNA end-trimming activity critical for DNA repair. Molecular cell. 2021, 81(7), 1534-1547.
16. ZADEH, FiroozehAbolhasani, et al. Cytotoxicity evaluation of environmentally friendly synthesis Copper/Zinc bimetallic nanoparticles on MCF-7 cancer cells. RendicontiLincei. ScienzeFisiche e Naturali, 2022, 1-7.
17. ROHMAH, Martina Kurnia, et al. Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant capacity and serum and mucus biochemicals in the common carp, Cyprinuscarpio exposed to abamectin. Fish & Shellfish Immunology, 2022, 129: 221-230.
18. ARIF, Anam, et al. The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. International Immunopharmacology, 2023, 114: 109581.
19. MARGIANA, Ria, et al. Functions and therapeutic interventions of non-coding RNAs associated with TLR signaling pathway in atherosclerosis. Cellular Signalling, 2022, 100: 110471.
20. H. A. Al-Hchaimi, M. F. Alhamaidah, H. Alkhfaji, M. T. Qasim, A. H. Al-Nussairi and H. S. Abd-Alzahra, "Intraoperative Fluid Management for Major Neurosurgery: Narrative study," 2022 International Symposium on Multidisciplinary Studies and Innovative Technologies (ISMSIT), 2022, pp. 311-314, doi: 10.1109/ISMSIT56059.2022.9932659.
21. MOHAMMED, Zainab; QASIM, Maytham T. The Relationship between Insulin Resistance and Hypertension in Patient with Hypertensive. HIV Nursing, 2022, 22.2: 1659–1663-1659–1663.
22. LEI, Zimeng, et al. Detection of abemaciclib, an anti-breast cancer agent, using a new electrochemical DNA biosensor. Frontiers in Chemistry, 2022, 10.
23. BASHAR, Bashar S., et al. Application of novel Fe3O4/Zn-metal organic framework magnetic nanostructures as an antimicrobial agent and magnetic nanocatalyst in the synthesis of heterocyclic compounds. Frontiers in Chemistry, 2022, 10.
24. LAFTA, Holya A., et al. Tumor-Associated Macrophages (TAMs) in Cancer Resistance; Modulation by Natural Products. *Current topics in medicinal chemistry*, 2023.
25. AL-JASSANI, Mohammad J., et al. Isolation and Evaluation of Antibacterial Agents Produced by Soil Bacillus SP. and Study Some of their Immunological Parameters. *Revista Electronica de Veterinaria*, 2022, 23.4: 105-111.
26. SANE, Shahryar, et al. Investigating the effect of pregabalin on postoperative pain in non-emergency craniotomy. *Clinical Neurology and Neurosurgery*, 2023, 226: 107599.
27. Zhao, Z., Shang, P., Sage, F., &Geijsen, N. Ligation-assisted homologous recombination enables precise genome editing by deploying both MMEJ and HDR. Nucleic Acids Research, 2022.