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Evaluation of Direct and Nested PCR for the Detection of Hepatitis B Virus in Chronic Carriers and Asymptomatic Blood Donors

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Annotation: Severity of HBV contamination is directly associated with immunological responses in the host, both those of an antibody and cellular nature, whereby immunological responses are a key public health burden. In this study, the properties of virus genetics responded to immunity and clinical stage in chronic HBV patients of Iraq compared to healthy HBsAg-positive patients. The researchers also wanted to demonstrate that immunological status was significantly associated with liver disease severity and duration of disease. Finally, sixty healthy participants and sixty others with chronic hepatitis B type B in Nasiriyah city between June 2014 and February 2016 were included in this study in liver and gastrointestinal disease centers at the University Hospital and Thalassemia Centre. The average age of chronic hepatitis B patients has persisted in rising and reached 64.5 years in February 2024–2025. The study found that the healthy HBsAg youngsters were, on average, 35.1 years old. Men were 13.6 times as likely as women and 12.2 times as likely as men with chronic conditions to have the condition.

Gender inequality has consequences for population health. Nested PCR amplification method revealed that the genetic material present in the samples investigated (both sick and asymptomatic carriers) was DNA. Positivity of PCR was 30.0% in chronic disease patients and 46.7% in healthy carriers. Clear, clean, yes, separating out the two concurrent conversations. The identification of hepatitis B virus genetic material (HBV DNA) in Iraqi patients with chronic hepatitis B by nested polymerase chain reaction (nested PCR) shows greater sensitivity and informativeness. The latest recommendations issued in Iraq for diagnosing viral hepatitis include screening for viral load, coupled with nucleic acid testing and RT-PCR.

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

Introduction

Hepatitis presents with characteristic histological and immune features. (Hepatocellular necrosis is defined as the death of hepatocytes and is followed by the infiltration of inflammatory cells into the liver, which is the event leading to liver pathology. Clinically and pathologically, the term "hepatitis" encompasses many liver diseases that can occur in parallel (St. Louis, 2014). A series of very toxic chemical agents can seriously challenge the integrity and function of hepatocytes, resulting in the development of inflammation, which can result in the expansion of hepatitis (Girish et al., 2008; Malaguarnera et al., 2012). Some of these chemical agents, which are present in the environment, include viral, bacterial, and fungal infections, as well as the use of agents such as alcohol and tobacco. Hepatitis, which is inflammation of the liver, is essential for the normal operation of the human body and is caused by various liver diseases. There are at least five separate viruses that differ markedly in their ecology, immunology, and epidemiology, as pointed out by Webb et al. in their 2020 study. But even with headway in medical science, viruses are still the enemy No. 1—and the most common cause of hepatitis in people. You also need to bear in mind that viruses still represent the major means of transmission even today. The several and varied types of viral hepatitis are as follows, in no specific order: B, also referred to as Acute hepatitis; C, also referred to as After a plasma transfusion.

Delta hepatitis, referred to as non-A, non-B, and non-D hepatitis, is also transmitted by the fecal-oral route, as is hepatitis E. Infectious hepatitis is also associated with the first letter of the

alphabet, further underscoring its importance, which is an ailment that impacts the liver and is spread primarily through the consumption of water or food that has been tainted by the hepatitis A virus. The cited books are Arankalle (2008), Dienstag and Isselbacher (2005) and once more Arankalle (2010). A disease is often referred to as cryptogenic when there is suspicion that its cause is due to the presence of some virus that has yet to be properly classified or recognized as such by the scientific community. Often, people presenting high liver enzyme levels for over 6 months are often thought to be suffering from chronic hepatitis, but their presenting signs and symptoms are the same as those of multiple inflammatory conditions (Chowdhury et al., 2004; Tajiri et al., 2022; Takaura et al., 2022). Persistent hepatitis B virus (HBV) infection has a catastrophic impact on the lives of over 400 million people worldwide and presents a significant long-term health risk. To effectively combat this illness, it is essential to prioritize proper disease management and prevention, both of which are critical components. Ten percent of individuals with chronic hepatitis B virus infection may progress to liver cirrhosis, a severe condition impacting liver function. Moreover, it is estimated that just fifty percent of these individuals would survive five years post-diagnosis of the illness. A study done in 2004 and published by Horvat et al. and Weissberg et al. indicated that human papillomavirus is responsible for two hundred thousand cases of liver cancer each year in various places worldwide.

Iraq has an endemic population of hepatitis B virus (HBV) infections, which imposes an extra burden on the healthcare system. According to many studies, including those by Jawad et al. (2018) and Tarky et al. (2018), Iraq is categorized as a country with moderate HBV prevalence. The seroprevalence of hepatitis B in the general population exhibits considerable fluctuation, ranging from 2% to 4%. As per the recommendations set out by the Centers for Disease Control and Prevention (CDC) in 1999, all children should get vaccination against hepatitis B infection before birth in countries with high endemicity of the disease. This is true irrespective of the level of danger the child presents.

In vitro polymerase chain reaction (PCR) may be used to amplify a specific segment of deoxyribonucleic acid (DNA) (Newton and Graham, 1997; Kaneko et al., 2000; Bermingham and Luettich, 2003; Fakruddin et al., 2013). This approach has gained popularity in recent years. To achieve this objective, this is one of the several methods that may be used. Oligonucleotide primers are necessary for DNA replication via PCR. Primers, which are small single-stranded DNA molecules, are designed to complement the DNA template sequence at its terminus. Extended primers are to undergo elongation by DNA polymerase on the template, specifically single-stranded denatured DNAs, in the presence of deoxynucleoside triphosphates (dNTPs), after the reaction conditions have reached their optimal state. Numerous investigations (Huang et al., 2022; Tortajada-Genaro et al., 2022; Van Cauwenbergh et al., 2022; Szokoli et al., 2022) have shown that the execution of this technique yields new DNA strands that are complementary to the templates. Upon the exit of these strands from the cell at this specific moment, a double-stranded DNA molecule is generated. Employ DNA polymerase at the appropriate temperature for enzymatic processes to elongate the primers, and heat-denature the double-stranded DNA to facilitate primer annealing (Shiromoto et al. 20, 2021). The subsequent stage involves doing another round of strand synthesis. Furthermore, investigations were carried out by Siki et al. (2005) and Sili et al. Viral DNA may be identified during the first phases of antiviral treatment, as noted in 2014. The giving of antiviral medicine to a patient does not affect the diagnostic process; thus, this is the right reaction. It is possible that low-level HBV viremia, which may be diagnosed by HBV PCR, can be discovered using the traditional hybridization approach for persons who are experiencing persistent liver sickness and who have a positive HBsAg test but a negative HBV DNA test. This is due to the fact that HBsAg is a marker that is typical of HBV. The evaluation of antiviral drugs, the assessment of HBV infection after liver transplantation, and the detection of HBsAg in acute or chronic liver disorders are all areas in which this is beneficial (Saiki et al., 2005; Pollicino et al., 2012; Sili et al., 2014). These are the areas in which an advantage may be gained from it. Simply said, these are only a few instances of situations in which it would be

advantageous to proceed. This tactic is quite useful in a wide variety of situations, including those that happen to each person on their own. According to Kaneko et al. (2000) and Pourazar et al. (2005), the detection of HBV-DNA in serum, tissues, and mononuclear blood cells may currently be accomplished by the use of standard hybridization techniques. For the purpose of detecting HBV-DNA, which is the most direct and sensitive indication of viral spread, a semiquantitative liquid hybridization approach has been designed. As stated by Siki et al. (2005) and Lau et al. (2011). There have been studies conducted on exactly these kinds of assessments. Blood donors or a history of HBV infection by women who are positive for anti-HBe antibodies but negative for HBV DNA in serum (Nishimori et al., 2016; Kafeero et al., 2022) are two of the factors that might be considered. On the other hand, this is because of the latter reasons that are described in the following, which support the need to design even more sensitive tests to detect HBV-DNA sequences. Both Kafeero et al. (2022) and Nishimori et al. (2016) are the authors of the papers that are being considered for inclusion in this article. When it comes to monitoring these patients who are receiving antiviral medication, it is of the utmost importance to ascertain whether or not they have had a partial or complete response, or even reactivation. However, it is impossible to prevent the exact order in which the amplified products are arranged in a genetic laboratory. In order to achieve such an ordered sequence, the Polymerase Chain Reaction (PCR) technique is required. According to Logoida et al. (2022) and Nabi et al. (2022), even though negative test results for HBV-DNA in the blood as well as for HBV-DNA and HDV have been obtained, there is no evidence found for autoimmune liver diseases and all other potential causes of liver damage in CEE patients who continue to be HBsAg- and anti-HBe-positive. Additionally, in CEE patients who test negative for serum HBsAg, there is a possibility that some patients with CAH may still have evidence of ongoing liver disease. In certain individuals, the clinical condition is like this. These patients have chronic active hepatitis, which is an illness that requires specialized monitoring and therapy. In individuals who have acute or chronic liver illness and have a negative test for HBsAg, there is a possibility that they contain hepatitis B virus (HBV) DNA in their blood, liver, or mononuclear cells. In research conducted by Ali et al. (2022) [11], it was discovered that donor blood samples contained HBV-DNA, despite the fact that hepatitis B virus (HBV)-specific serological tests were negative. An extended PCR potential was demonstrated in a highly sensitive hepatitis B virus (HBV) DNA detection in the latest work that was conducted by Poortahmasebi et al. (2022), Chen et al. (2022), and Pacin-Ruiz et al. (2022).

Objectives of the study:

With this study, the researchers had two aims they wanted to achieve:The first was to study if certain HLA class I alleles are associated with disease pathogenicity, which means that you just do not get better, and the second was to study whether these same alleles are associated with disease carrier rate. In this paper, methods of direct and nested PCR for HBV DNA detection and the similarities and differences will be reported. Both people with chronic hepatitis B and those who are healthy but have the virus can pass it on at the same time. HBIG and PCR HBV may also be identified using the PCR assay.

Methods and other materials

Directed against the HLA Class I target, the immunotherapeutic test works when a patient is positive for this. Groves et al. (2022) reported that a nucleic acid test (NAT) was performed. PCR was employed to detect the HBV DNA. Hu et al., in 2022. PCR methods make it possible to detect HBV DNA.

The viral core region that can be amplified by the AB analitica kit includes the target for detecting HBV DNA. Examining the whole notion

Three separate nucleic acid segments must participate for the technique to work. In this process, a pair of single-stranded oligonucleotides, called primers, anneal to the target DNA, a single-stranded template containing double-stranded DNA, and amplify the target DNA.

In the region of the primer, there is a molecular release primer or the DNA polymerase whose activity promotes the linking and joining of complementary dNTPs (free nucleotides) present in solution. This is achieved by starting the process of synthesis. In this way, the DNA polymerase can replicate the region of DNA of interest to form new double-stranded DNA molecules, which are indistinguishable from the original DNA molecules. In a study published in 2022 by Hong et al., millions of such DNA molecules can be obtained in a process that requires multiple rounds of cycling.

Noting the primer Ordered-for sequence is definitely something to be done. The 5′-TGTGACGACTGAGGTAGAAG-3' kit for the detection of HBV DNA was supplied by the Italian business AB Analitica.

Commonly, a relative risk, or RR, is used to quantify the measure of association Present between a genetic marker and a disorder. In this context, the statistic in question indicates the risk (or relative risk) in sick persons of having markers compared with being marker-free. A formula that may be used to derive the RR when attempting to specify the variable RR is:

RR = $(a \times d)/(b \times c)$

The aggregate of all patients who passed the marker.

c. Count of persons with negative antigen test results.

Total number of inspections favoring the marker.

A correlation coefficient greater than one indicates a positive link between the variables, whilst a value less than one denotes a negative relationship between them. A combination of capital and lowercase letters was used to represent the etiological fraction, sometimes referred to as the thermometer, which indicates the degree to which the condition is authentically attributed to the specified ingredient. Given that EF is a kind of trust that remains steadfast in relation to any individual or element, the following formula is the most efficient method for its decryption:

EF = (RR-1)/RR × a/(a+b)

The percentage of sickness that can be avoided as a result of the disease-associated marker in the first scenario is referred to as the preventive fraction (PF). The formula below, which specifies PF, is as follows:

PF = ((1 - RR) * (a / (a + b))) / (RR * (1 - (a / (a + b))) + (a / (a + b)))

The two factors, EF and PF, are both variables; each can be any value between zero and one (zero means no connection and one means the strongest connection). Two-by-two contingency tables are created for the four preceding cumulative counts (a, b, c, and d). The significance levels, as an index of probability, are subsequently calculated using Fisher's exact probability (p). Svejgaard et al. (1983) discovered that the corrected probability (Pc) can be calculated by using the number of antigens examined at each HLA locus multiplied by the probability (P). This was in order to avoid an accidental association that was reached, given the myriad of comparisons that were induced.

Results

Nucleic Acid Test (NAT).

Molecular diagnosis of HBV DNA in different situations has been created in these chronic patients and the carrier group. This is especially so of the Nested PCR assay. Recent investigations have indicated that there is a very high correlation between the PCR direct method and the Nested PCR method ($P<0.001$). With the PCR direct approach, 30% of chronic and 47% of carriers were HBV DNA positive. The Nested PCR, on the other hand, was one hundred percent positive for HBV DNA in both groups (Table 2 & Figs. 1 & 2).

Table 1: The following delineates the differences between the chronic group and the carrier group as identified using different PCR methodologies.

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,	14	23	37,
	Inside the case%	37.83%	62.16%	100%
Total	Account	29	31	60,
	% Inside the case%	48.33 %	51.66 %	100 %
Nested polymerase chain reaction for hepatitis B virus	Positive Count	15,	22,	37,
	Inside the case%	40.53,%	59.47,%	100,,%,
Total	Amount	13,,	10,,	23,,
	Inside the court case %	56.51,%	43.48,%	100,,%

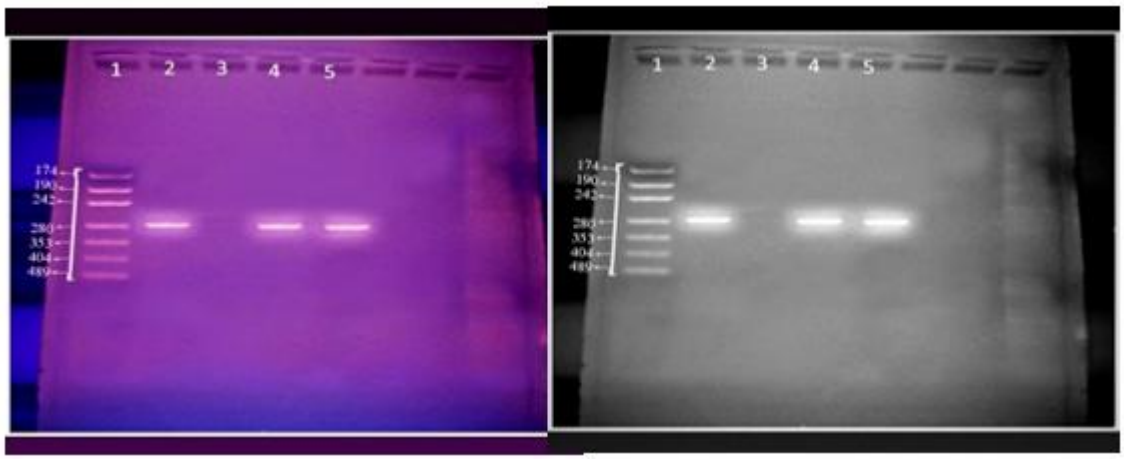

Figure 1. DNA extraction was performed as previously described [12], followed by electrophoresis on a 3% agarose gel (direct amplification). with detection of HBV DNA following 45 minutes at 100 volts. The first DNA marker is, for example, the first. The second choice is an HBV positive control. 3) Sample that goes into self-destruct mode (gives +ve HBV 270 bp)

Detection of HBV DNA: 45 min at 100V in a 0.3% agarose gel following amplification in a normal technique. Results can be observed in Figure 1. 1) Something to identify DNA. 2) A positive health report of HBV. 3) Hinting at deleting for itself a HBV positive isolation (270 bp band).

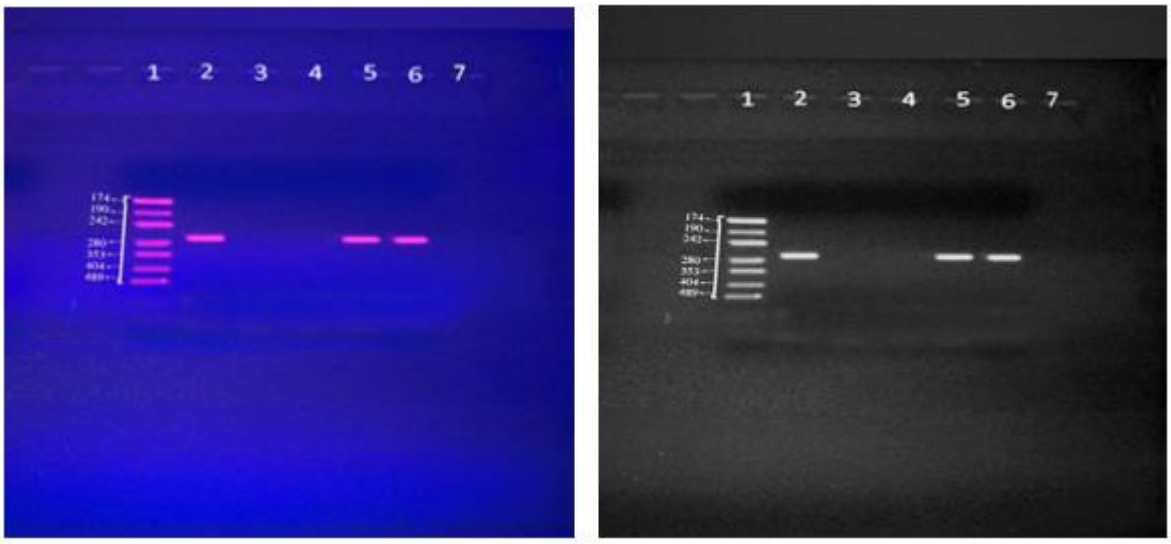

Figure 2: 3% agarose gel electrophoresis (nested amplification) carried out at 100 volts for 45 minutes to identify HBV DNA. The main DNA marker. The best option is the positive control for HBV. The third method is the positive control of direct amplification. 4. Using a negative control sample that tests positive for HBV, the regulation of nested amplification (5 and 6) (258 bp band).

Discussion

According to Brown et al. (2022), approximately 5% of the world population is considered a chronic carrier of HBV. Incidence of chronic hepatitis B infection is a common ailment throughout the world. Furthermore, the disease is considered endemic in Iraq and is also frequent in a large number of other poor countries (Moonsamy et al., 2022). This study considers numerous parameters, including virological, immunogenetic, and molecular factors that are likely to be correlated with infection. In this experiment, study participants included fifty CHB cases and fifty HBV carriers. Indeed, age at the time of detection of infection seems to be the most important factor for projecting prognosis. The mean age of the patients is the average age of the population. The disease was found to be a sexually transmitted disease and was determined to be between 24-36 years and 36 years old. and the average age of disease-carrier individuals was 35.14–45.06 years for chronic patients in this study. Kumar et al. (2022) reported a mean age of CHB patients to be 45 years, which was also in line with other studies carried out in Iraq. The work of Al-Fraiji and Al-Shammary (2022) revealed that the mean age of the carrier group was 38 years. On the other side, Aghdaie et al. (2022) revealed that the carrier group's mean age was 37 years, compared to 42.6 years for the chronic group. This was quite a change.

Aghdaie et al.'s findings in 2022 indicated that the mean age of the CHB patients was higher than that of the carrier group. This could well be due to an earlier than normal age at which people are being exposed to HBV. Of those who are chronic, the male-to-female ratio is 2.2:1; of those who are carriers, 3.6:1. This might mean that they depict more men than women. In contrast, it is possible that males are also more prone than females to be exposed to HBV risk factors. Men might be more at risk of alcohol consumption, which could exacerbate HBV-associated liver injury (Zahn et al., 2021). One of these two plausible reasons may clarify the disparity of gender differences between the two subgroups. Actually, hepatitis B infection is a recognized cause of chronic liver disease worldwide. several diverse factors are also responsible for the multiple modes of transmission of HBV; some of these are still controversial. The practice has many different aspects behind it. The polymerase chain reaction (PCR) is performed in vitro, a technique commonly known as PCR, used to amplify a defined fragment of deoxyribonucleic acid (DNA) (Zhao et al., 2022). HBV DNA in peripheral blood and liver can be detected with polymerase chain reaction (PCR), which has been widely used with favor in recent years. Known as PCR, this polymerase chain reaction allowed scientists to differentiate the very small amounts of nucleic

acid found in the patient's blood. Jackson et al. (2022) maintain that the detection of DNA of the hepatitis B virus is a sound indication of the presence of the virus and subsequent replicative action. It is also used to determine whether a patient who has received a liver transplant has HBV infection, whether antiviral medication is effective, and whether HBsAg is present in acute or chronic liver disease. All these are instances of how this method can be applied in various situations.

The development of molecular methods for HBV DNA detection in chronic and carrier individuals has been bringing about a clearer picture of infection, particularly with the use of Nested PCR (Figure 1). That is even more so now with these technologies coming in. The findings presented here indicate that the PCR direct procedure is a more sensitive method for detecting HBV DNA. This is not the case, as opposed to the nested PCR methodology (See above). Using the direct method, the positive rate for the carrier group was 46.7% and that for the chronic group was 30.0%. In contrast, the Nested method had 100% positive results for HBV DNA in both groups.

Other investigators showed that HBV DNA was detectable in 44.0% of HBV carriers and 37.0% of CHB patients. It was done through direct amplification of HBV DNA, with a detection limit of 8,600 copies/mL (Tian & Zhao, 2004). In addition, using a commercially available PCR assay, HBV DNA could be detected by Pawlotsky (2008). In CHB patients, 56.0% of results were positive and 48.0 % in HBV carriers, using a limit of detection of 6.340 copies/mL HBV. "They were the ones who found the good results.

Barlet et al. (2001) observed HBV DNA in a high prevalence of carrier HBV and the majority of CHB samples. This was accomplished with a nested method at a concentration of 560 copies/mL of low HBV DNA. This was found even though the CHB samples exhibited the highest concentration of antibody. In the research performed by Ezea et al. (2022), an asymptomatic HBV carrier and a chronic HBV carrier were both HBV DNA-positive in 92% and 89%, respectively, with a nested polymerase assay. The sensitivity limit of HBV DNA detection was 400 copies/mL. Additionally, Gionda et al. (2022) found few differences between the groups concerning the nested PCR protocol.

This research made an in-depth comparison between the similarities and differences in two newer molecular methods for detecting hepatitis B virus (HBV) DNA. The results of the study have demonstrated that the second round of molecular polymerase chain reaction testing was very sensitive in detecting and distinguishing impaired hepatitis B virus deoxyribonucleic acid present in the blood sample of the subject. This method is therefore considered the most efficient and suitable to solve the problem in a global form. In conclusion, information provided in this document incontrovertibly demonstrates the health impact and utility of this advanced molecular test that has brought about a notable improvement in the likelihood of identifying disease early and impeding the progression of liver disease and HCC to later stages. Because the polymerase chain reaction (PCR) is highly sensitive and specific, it can be used to detect the virus early after infection, well before the onset of symptoms. I'd also stress that the identification of early disease may have an important role for health professionals in managing the disease. To end, I will just throw in a couple of additional observations to deepen our discussion. There is a lot to think about before the deciding vote. It's also a time for consideration of our actions over the long haul. In other words, we need to keep our minds open and understand how nuanced the issues are. Thanks, Alezi, these closing words have been precious for me. Most of the modern molecular techniques with high technology for the complete and detailed detection of the hepatitis B virus DNA, in cases of chronic patients and carriers have recently advanced considerably in a way that has made it feasible to capture the whole virus imagery upon the body. This figure gives a broad picture of the situation, especially concerning the nested polymerase chain reaction (PCR) protocol. Modern academia has indicated that the nested polymerase chain reaction (PCR) strategy is much more sensitive than the conventional direct PCR technique used as a method to accurately detect the genetic characteristics of the HBV. It was properly deduced, in the light of the painstaking and

ample data, through logical processes, with reasonable inferences to the corollaries of truth from which no reasonable man would dissent.

Conclusions and final remarks on the issue of the present document.

The significance of the above-mentioned antiviral process to HBV infection is based on the body's capacity to launch an adequate immune response, a factor that presents a paramount challenge to public health aspects due to the communication between humoral and cellular immunity. The comprehensive investigation methodically examined the clinical conditions of Iraqi patients with chronic hepatitis B in comparison with persons who are HBsAg positive and healthy. Further, virus properties and genetic immunologic responses were analyzed in detail in the two investigated groups. The objective of the study was to establish that liver disease severity, not only its duration, was highly associated with the immunological status of the enrolled patients. The sample size was selected as 60 healthy HBSA carriers versus 60 patients with a previous diagnosis of type B chronic hepatitis B from distinguished health service centers in southern Iraq, including the Iraqi Central Blood Bank, Thalassemia Center, and University Hospital for Gastrointestinal and Liver Diseases, who had appropriate and timely medical interventions. Men were 12.2 times more likely than women to develop chronic diseases and 13.6 times more likely to live with them. With respect to the amplification process, these observations definitely proved that the genetic material contained in the samples analyzed, regardless of whether they were from chronic patients or asymptomatic carriers, was entirely made of DNA. Healthy carriers were found with a rate of 46.7%, observing a 30.0% incidence in the PCR-test results. In the very particular stage that was minutely investigated, it was relatively easy to faithfully identify and differentiate the two main descriptions in progress. The utilization of nested polymerase chain reaction (PCR) within the country is shown to be very efficient for emphasis on accuracy for detecting hepatitis B virus (HBV) infection in subjects with chronic hepatitis C and for maximum utilization of viral hepatitis research laboratories in Iraq, the authorities should enforce the application of highly sensitive nucleic acid testing, accurate measurement of the viral loads, and real-time PCR based technology according to the most recent and stringent regulations that have been implemented.

Thereby, we think that the application of modern diagnostic methods by using special tools in the genetic and molecular fields plays an important role in the diagnostic processes, in addition to using computer techniques and electronic computing in building diagnostic systems applicable to artificial intelligence systems related to medical, epidemiological, and viral disease fields.

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