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 City southern 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.

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 similar clinical features [5]. More than 400 million people worldwide are chronically infected by HBV, which is a very long-term health problem and at least 10% of chronically infected individuals develop cirrhosis and five year 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 of intermediate hepatitis B endemicity among normal Iraqi population which is a public health problem, and the outcome of infection depends on the strength of the natural and conditioned response of humoral and cellular immunity. This study was conducted to evaluate the relationship between the immune status and the chronic condition of the disease. 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.
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:

\[
RR = \frac{a \times d}{b \times c}
\]

\[a: \text{Number of patients positive for the marker.}\]
\[b: \text{Number of patients negative for the marker.}\]
\[c: \text{Number of controls positive for the marker.}\]
\[d: \text{Number of controls negative for the marker.}\]

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:

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

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:

\[
PF = \left(\frac{1 - RR}{RR}\right) \times \left(\frac{a}{a + b}\right)
\]
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 [17].

<table>
<thead>
<tr>
<th>Nucleic Acid Test</th>
<th>cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>healthy carrier HBV</td>
<td>chronic HBV</td>
</tr>
<tr>
<td><strong>PCR direct amplification of HBV DNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Count</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>% within case</td>
<td>56.52%</td>
<td>43.48%</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>% within case</td>
<td>40.54%</td>
<td>59.46%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>% within case</td>
<td>46.67%</td>
<td>53.33%</td>
</tr>
<tr>
<td><strong>Nested PCR of HBV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Count</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>% within case</td>
<td>40.54%</td>
<td>59.46%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>% within case</td>
<td>56.52%</td>
<td>43.48%</td>
</tr>
</tbody>
</table>

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

**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-Fraiij, 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].

Regarding 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 sequence from a target DNA sample. The PCR process involves a series of temperature cycles that include denaturation (heating to separate the DNA strands), annealing (cooling to allow primers to hybridize to the target DNA), and extension (heating to allow DNA polymerase to synthesize new DNA strands). This cycle is repeated multiple times, resulting in the exponential amplification of the target DNA sequence.

**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 bp band).
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 direct protocol 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 percentage 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 et al. 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 (560 copies/mL). BV samples by nested protocol with low level of HBV DNA (560 copies/mL), behind that Ezea, et al. 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.

**Funding**

Self Funding

**Acknowledgement**

For all the worlds to research

**Conflict of Interest**

No Conflict of Interest

**Inform Consent and Ethical Statement**

Not Required

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