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Original Research Article

Extending the Conventional Method to Hepatitis B Virus: A Molecular Detection and Diagnostic Study

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Abstract: Despite a global immunization campaign, hepatitis B virus (HBV) infection remains a severe public health risk. The study advances our understanding of the diversity and evolution of the hepatitis B virus by studying virus genotypes using PCR and sequencing. This insight will be extremely useful in the development of targeted medicines and immunizations to combat HBV infection. The results of HBV testing on twenty samples, three of which tested positive and the remaining 17 were negative. A fast test, however, yielded positive results, indicating a mutation that hampers diagnosis in the detection of a 492 base pair band. The HBV sequences (seq1) were observed to mimic strains from other countries, with four SNPs causing a gap between seqs 1 and 2. It was also stated how many people in China who donated blood tested positive for HBV DNA. According to the phylogenetic tree analysis, sequences 1 and 2 were 40% related to one another in an international strain, whereas sequences 3 and 4 were independently classified and shared 41% similarity, implying that just a fraction.

Keywords: Hepatitis B, PCR, phylogenetic analysis, HBVs.

INTRODUCTION

The 10th greatest cause of death globally is the hepatitis B virus (HBV), a severe public health issue. Globally, this virus affects roughly 2 billion people, of whom 350 million have a chronic illness. Around 600,000 individuals per year pass away from the acute or long-term effects of hepatitis B (Lavanchy, 2004). Sumeria in the third millennium B.C. is where the earliest recorded case of jaundice in the history of hepatitis is found. Its transmissibility was first identified in 1494, when syphilis was inadvertently spread by Columbus and his crew. Outbreaks occurred after immunizations with human serum or lymph; the greatest among personnel in the US Army occurred in 1942. In the 1940s, a virus was identified as the cause of hepatitis; two distinct forms of viral hepatitis were identified in the late 1950s and early 1960s. The discovery of the Australia Antigen (Au Ag). As a result, the Au Ag was designated as hepatitis B surface antigen (HBsAg). Core nanoparticles were released from these "Dane particles" by Almeida *et al*., using mild detergents (Kramer, 1963).

The response to these inner/core particles came in 1972. Robinson *et al*., detected hepatitis B virus (HBV) DNA at the same time. The Complement Fixation Test was once used to demonstrate an antibody titer, which allowed for the detection of HBV infection. Ling *et al*., at Abbott Laboratories created Ausria 125, the first solid-phase sandwich radioimmunoassay. Hepatitis B virus (HBV) DNA integration is an uncommon event that happens in fewer than 1% of infected hepatocytes during viral infection. On the other hand, HBV DNA is the least prevalent somatic mutation and is present in the genome of approximately 90% of HBV-related hepatocellular carcinomas (HCC) (Chisari, 2013). Those with chronic hepatitis B exhibit random integration in their human chromosomes, but those with hepatocellular carcinoma exhibit genomic integration hotspots. This observation implies a robust positive selection favoring the progression of HBV-integrated hepatocytes to HCC, resulting in an HBV-integrated cell enrichment in HCC.

There are drawbacks to the current approaches for diagnosing HBsAg, including the requirement for

costly equipment and skilled personnel. Thus, a method for the sensitive, specific, quick, easy to use, and economical analysis of tiny biological samples is required. Biosensors, which provide real-time, label-free, and affordable analysis, have become an excellent tool for identifying and evaluating biomarkers. Biosensors can now be used for point-of-care testing because to advancements in technology, which allows for prompt diagnosis and patient care (Kumar *et al.*, 2023).

MATERIAL AND METHODS

Twenty hepatitis B virus-infected patients' blood samples were collected. A commercial DNA extraction kit (QIAGEN) were used to extract the viral DNA from the blood samples.

PCR amplification with specific primers Forward primer GGGGGCTGTATTTTCCTGCT and reverse primer GAGTGGTGCAGGTTTTGCAG with product size 492 were optimized at 58C annealing temperature to amplify a part of the hepatitis B viral genome. The results of the PCR were then presented on an agarose gel to confirm the presence of the amplified DNA fragment.

The amplified DNA fragment sequenced using Sanger sequencing technology. Phylogenetic analysis: Using bioinformatics techniques, compare the obtained sequences to reference sequences from different hepatitis B virus genotypes. The genotype and subtype of the hepatitis B virus must be determined by phylogenetic tree y bootstrap analysis using Mega 6 software UPGMA method, assuming the mutation existent.

RESULT AND DISCUSSION

Three of the twenty samples were verified to be positive, with the remaining specimens being negative. Nonetheless, the quick test produced positive findings for HBVs, demonstrating that the virus's genome exists genetically and that the negative results are due to a mutation in the genome that impacts diagnosis. The band in Figure 1 depicts the standard band with right base pair 492. This result was accepted by In samples whose HBeAg status was determined by the AxSym HB sAg/eA test, the serum-based clinical sensitivity was 96.37% and the overall specificity was 99.37% (98.99% for serum and 100.00% for whole blood) (Shahid *et al.*, 2023).

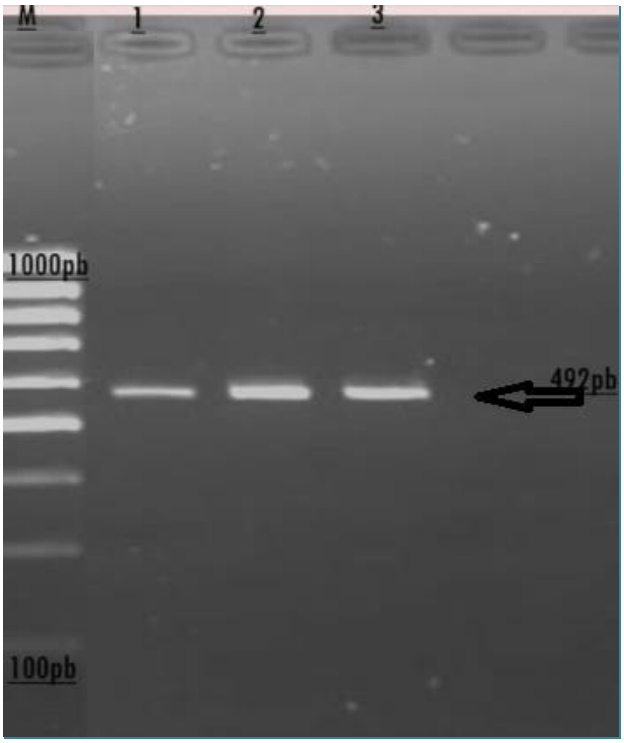

Figure 1: A band of 492 base pairs (pb) was detected on a 2% agarose gel electrophoresis after PCR amplification of the target gene

Even in the genome's deleted section, the HBV sequences (seq1) were identical to foreign strains, as shown by the alignment in Figure 2. Four single nucleotide polymorphisms (SNPs) were also present in seqs 1 and 2, creating a gap between the sequences that may have an impact on the severity of the disease as shown in the table 1.

More information reveals that three of the twenty samples had positive HBV tests, whereas the other samples had negative results. Conversely, the rapid test yielded positive results for HBV, proving the virus was present in the genetic material, while the negative results were probably caused by a mutation affecting the diagnosis.

According to Chao Liu *et al*., NAT screening was performed on 20,084,187 seronegative blood donors in China between 2010 and 2015. Out of all of them, 1/ 1,482 HBV DN (Chen *et al*., 2023). A-positive samples were discovered in seronegative blood donors, implying a detection rate of 0.0675% (6.75 per 10,000)]. According to our findings, there was one HBV DNA-positive blood donor for every 3195 (6-MP) in Jinan, Shandong Province, resulting in a detection rate of 0.031%.

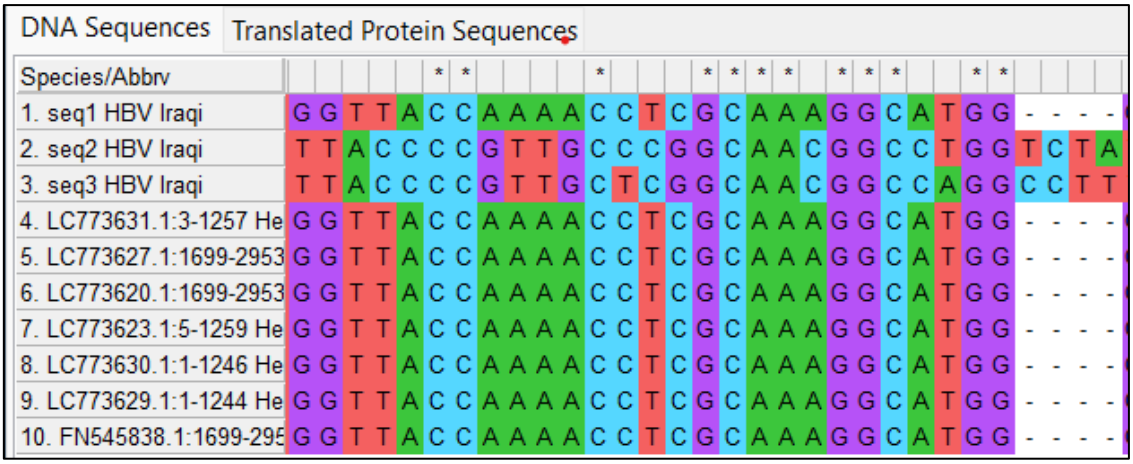

Figure 2: A comparison of the Iraqi virus with seven selected HBV isolates from the global Genebank indicated a high number of mutations

Table 1: Genetic variation and SNP of the HBVs Iraqi with seven selected HBV isolates from the global Genebank indicated a high number of mutations

No.	Species/isolate/strain	Identity (%)	Genetic variation	Type of muration
1	seq1 HBV Iraqi	100%	----	Deletion
2	Seq2 HBV Iraqi	100%	T----A A---G A----T C----G A----C	Transversions Transition Transversions Transversion Transversions
3	Seq3 HBV Iraqi	100%	T----A A---G A----T C----G A----C	Transversions Transition Transversions Transversion Transversions
4	LC773631.1:3-1257 Hepatitis B virus genotype A	100%	---	Deletion
5	LC773627.1:1699-2953 Hepatitis B virus genotype A	100%	---	Deletion
6	LC773620.1:1699-2953 Hepatitis B virus genotype A	100%	---	Deletion
7	LC773623.1:5-1259 Hepatitis B virus genotype A	100%	---	Deletion
8	LC773630.1:1-1246 Hepatitis B virus genotype A	100%	---	Deletion
9	LC773629.1:1-1244 Hepatitis B virus genotype A	100%	---	Deletion
10	FN545838.1:1699-2953 Hepatitis B virus	100%	---	Deletion

Sequences 1 and 2 were clustered together with an international strain, sharing 40% of the similarity in the phylogenetic tree. Sequences 3 and 4 were clustered separately, sharing 41% of the similarity. Despite this, the finding indicates that only a portion of the genome is conserved. According to the results of this study's HBV sequence analysis, which was conducted using the hepatitis B virus phylogenetic typing tool approved by (Jose-Abrego *et al*., 2023).

Seq2 could possibly be interpreted as a mosaic of Sequences Seq1 and Seq2 in the presence of recombination by applying the law of parsimony. The research discovered that the topological relationship exhibited by most phylogenetic trees of the three relevant sequences' matched sequence segments rooted triplet without reticulation, constituted the major phylogenetic relationship. According to the fundamental phylogenetic relationship, the ancestral sequence that gave the mosaic the most genetic content when compared to the other sequences is its progenitor. When there is a pool of primary rooted triplets, a consensus tree can be created to emphasize the essential evolutionary relationships of all feasible three-sequence combinations for multiple sequences (Jiang *et al*., 2013).

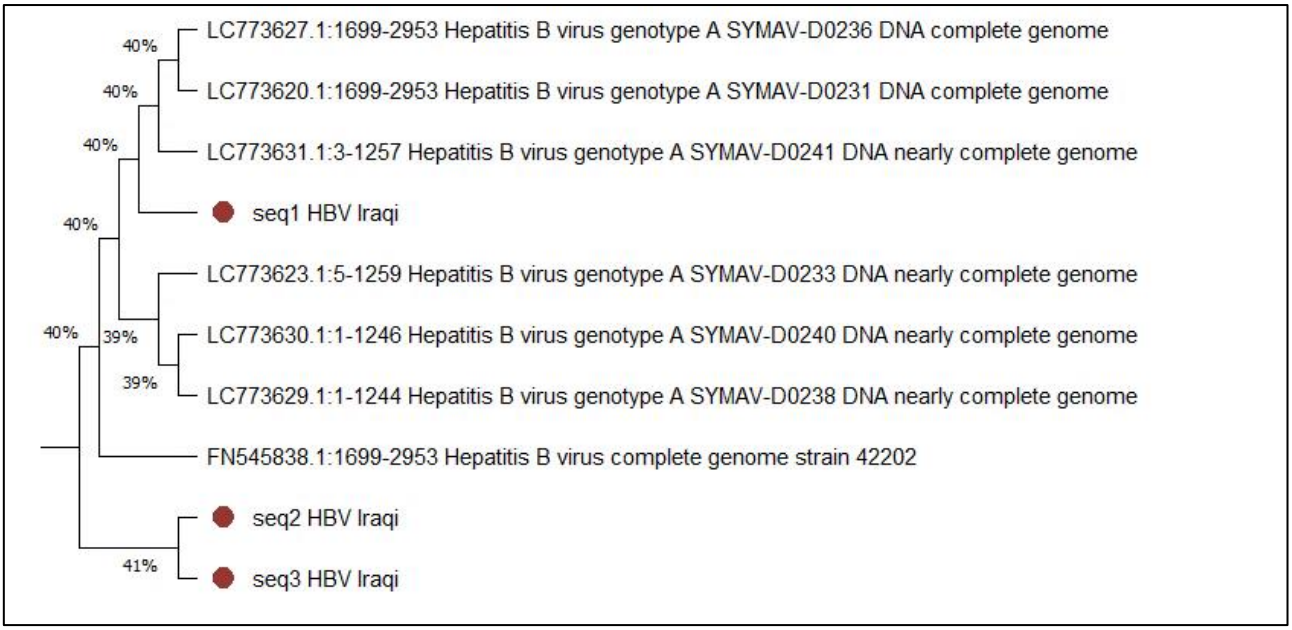

Figure 3: The genetic connect among the Iraqi hepatitis B virus strain and seven selected HBV isolates from the global Genebank is depicted in a phylogenetic tree generated in Mega 6 software using the UPGMA method with bootstrap 100 replicate for the analysis

CONCLUSION

Conclusions of the HBV test revealed a mixture of positive and negative results, with a quick test suggesting a possible mutation influencing diagnosis. Highlighted were the HBeAg test's clinical sensitivity and specificity as well as the discovery of a 492 base pairs band. In addition, the discussion of the detection rate of HBV DNA-positive blood donors in China, the resemblance of HBV sequences to foreign strains, and the existence of four SNPs causing a gap between sequences were brought up. Only a fraction of the genome is conserved, as shown by the clustering and similarity between sequences found in the phylogenetic tree study. It would take more investigation and analysis to reach thorough conclusions.

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