English Summry

Infection with the hepatitis B virus (HBV) leads to different disease outcomes and despite the availability of an effective vaccine Hepatitis B virus (HBV) infection remains a global public health problem.

Materials and Methods

1. Sample collection:

One hundred positive sera samples for the hepatitis B virus were collected from blood donation campaigns for the company blood transfusion services *(VACSERA)*. All patients were diagnosed HBV infection with no co-infection with other hepatitis viruses such as HCV, HDV, or HIV. All sera samples had been aliquot and stored at -20°C until use

2. DNA Extraction:

DNA of HBV was extracted and Purified according to the instruction of viral DNA extraction kit protocol.

3. Amplification of target genes of HBV (surface antigen gen and Core/ pre core region):

The Pre S region and the Core region were amplified using polymerase chain reaction (PCR) test by primer for each gene.

4. Detection of PCR Product:

PCR products were detected by electrophoresis in 1% agarose gel stained with Ethidiume bromide. The agarose gel was visualized using ultra violate (UV) to detect PCR product comparing with positive and negative

controls.

5. Recovery of PCR product from agarose gel:

After PCR products were separated by 1% agarose gel electrophoresis, PCR products were recovered from gel according to gel extraction kit protocol.

6. Hepatitis B surface antigen and Core/ pre core region Sequencing :

Amplified HBV DNA fragments were sequenced directly using the ABI Prism Big Dye Terminator V.3.1 Cycle sequencing Kit on an ABI 310 DNA automated sequencer (Applied Biosystems).

7. Removal of unincorporated:

DyeEx purification Kits had been used to remove all unincorporated dye terminator directly from sequencing reaction. The steps of removing the residual of dye were carried out.

8. Sequencing results and Bioinformatics analysis:

8.1. Preparing and Loading Samples for sequencing:

Sequencing had been carried out at Genetic Engineering Research Department (VACSERA). Electrophoresis process was performed on ABI Prism 310 Genetic Analyzer**,** by using ABI Prism 310 data base collection.

8.2. Sequencing assembly and Phylogenetic tree:

The sequence results generated by the forward and reverse sequencing primers were analyzed with the software program sequencing analysis 5.3.1

For sequence comparisons of Large s and Pc/core sequences of the eight HBV isolates retrieved from GenBank, sequence alignment was

performed using the multiple-alignment algorithm in Megalign (DNASTAR, Window version 3.12e).

Results can be summarized in the following

Three novel mutants of the hepatitis B virus surface antigen (HBsAg) were characterized. The mutants were isolated from asymptomatic patients who were found to be positive for HBsAg. The novel HBV isolate were clustered to genotype HBV/D to insure that the prevalence genotype in Egypt is genotype HBV/D. One isolate of HBV C/Core can be sequenced and it also clustered to genotype HBV/D.

This finding will allow the introduction of a new vaccine for HBV or help the emergence of a new diagnostic kit for HBV and to Control of these HBV mutants, who will require new drugs, vaccines, and treatment strategies, will become the next major challenge on the path to eventual elimination of HBV infection.