Genomic Profiling of Hepatitis B Virus and Hepatitis C Virus from 1 mL of Urine

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Abstract

- Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with a very poor prognosis. The main causes of Hepatocellular Carcinoma are the Hepatitis B virus (HBV) and the Hepatitis C virus (HCV).

- Early detection for such viruses is rare due to their silent behavior as well as the invasiveness of their detection methods. Detecting Hepatitis C virus and/or Hepatitis B virus from urine will facilitate their diagnosis as well as it will be useful for population screening especially for its non-invasiveness. It has been proved that circulating DNA resulting from apoptosis/necrosis will be rapidly cleared from the circulation to the urine through the kidneys (i.e. Trans-Renal DNA) and this DNA could be detected by PCR. Detecting Hepatitis C virus as well as the Hepatitis B pgRNA from urine samples is very challenging since RNA is unstable especially in such a biological fluid “Urine” with a high concentration of nucleases which could degrade the viral genome.

- In this study, urine samples were collected from patients with Hepatitis C virus and Hepatitis B virus at different stages of the disease as well as normal individuals. The DNA was isolated from 1mL of those samples using the Urine DNA Isolation Kit (Norgen Biotek)

- The RNA was isolated from 2 mL of those samples using the Total RNA Isolation Kit (Norgen Biotek).

- Preliminary data analysis for the DNA isolated from the urine samples revealed tremendous variability which can be helpful in understanding the mechanism of viral pathogenicity and the viral-host interaction. Hepatitis C virus was detected from the RNA isolated from the urine using specific Reverse transcriptase-PCR followed by PCR targeting different viral genes. Hepatitis B virus was detected successfully in urine in its DNA form as well as the pgRNA form.

- Sequencing of the PCR products revealed important information which can be very helpful in understanding the mechanism of the viral infection as well as predicting the outcome of the infection.

Introduction

- HCC is one of the most well-known malignancies worldwide. The number of cases of HCC is rising, and this is likely due to increased HCV and HBV infections (1).

- HBV is the most common and best investigated etiological agent of HCC, but the mechanism is poorly understood (2). HBV, HCV and HCC post HCV are known to alter the normal physiological function of liver cells.

- Early detection for HCV and HBV is rare due to their silent behavior as well as the invasiveness of their detection methods.

- Urine contains two different species of DNA: the larger species is generally greater than 1 kb in size and appears to derive mainly from the cells shed into the urine from the urinary tract, while the second species is smaller, generally between 150 and 250 bp, and derives mainly from the circulation, indicating that it has crossed the kidney barrier (3).

- Detecting Hepatitis C virus as well as the Hepatitis B pgRNA from urine samples is very challenging since RNA is unstable especially in biological fluids such as urine, which has a high concentration of nucleases which could degrade the viral genome.

- Most recent biomarkers research has been focusing on various forms of cancer due to the assumption that a growing cancer will affect the physiology of the organism to such an extent that measurable changes will be of a significant difference (3), thus allowing the detection of a proteomic pattern in biological fluids.

Methods

- Urine samples were collected from the Alexandria University General Hospital and the institute of Liver Diseases at El Monofya University (Egypt) upon the approval of the Brock University Research Ethics Board.

- 122 different urine samples were analyzed:
    - 16 from HBV infected patients.
    - 74 from HCV infected patients.
    - 32 from HCC post HCV patients.

- 10 urine samples from healthy Egyptian individuals were also used as a control for the genomic profiling.

- Total urinary DNA was isolated from 1 mL urine samples using the Urine DNA Isolation Kit (Norgen Biotek).

- Total urinary RNA was isolated from 2 mL urine from selected samples using Total RNA Purification Kit (Norgen Biotek).

- DNA samples (20 µL out of each 100 µL elution) were run on 2% agarose gels for qualitative analysis

- RNA samples (20 µL out of each 50 µL elution) were run on 1.5% denatured agarose gels for qualitative analysis

- RT-PCR was done to capture the HBV pgRNA using 4 µL RNA as a template superscript TM II Reverse Transcriptase (Invitrogen).

- RT-PCR was done to capture the HCV 5'NCR using 4 µL RNA as a template, 1 µL HCV 5'NCR primer 5' - GCCUUUCGCGACCCAA- CACU - 3' using the superscript TM II Reverse Transcriptase (Invitrogen).

- Amplification of different overlapping genes in the HBV viral genome was done by the multiplex PCR technique using 3 primers HBV2F 5'TTTACTAGTGCCATTTGTTCAGTG-3', HBV3F 5'GCTCCAGTTCAGGAACAGTAAACCC-3' and HBV3R 5'-ATTGAGATCTTCTGCGACGCGGCGA-3' using the Expand Long template PCR system (Roche).

Results

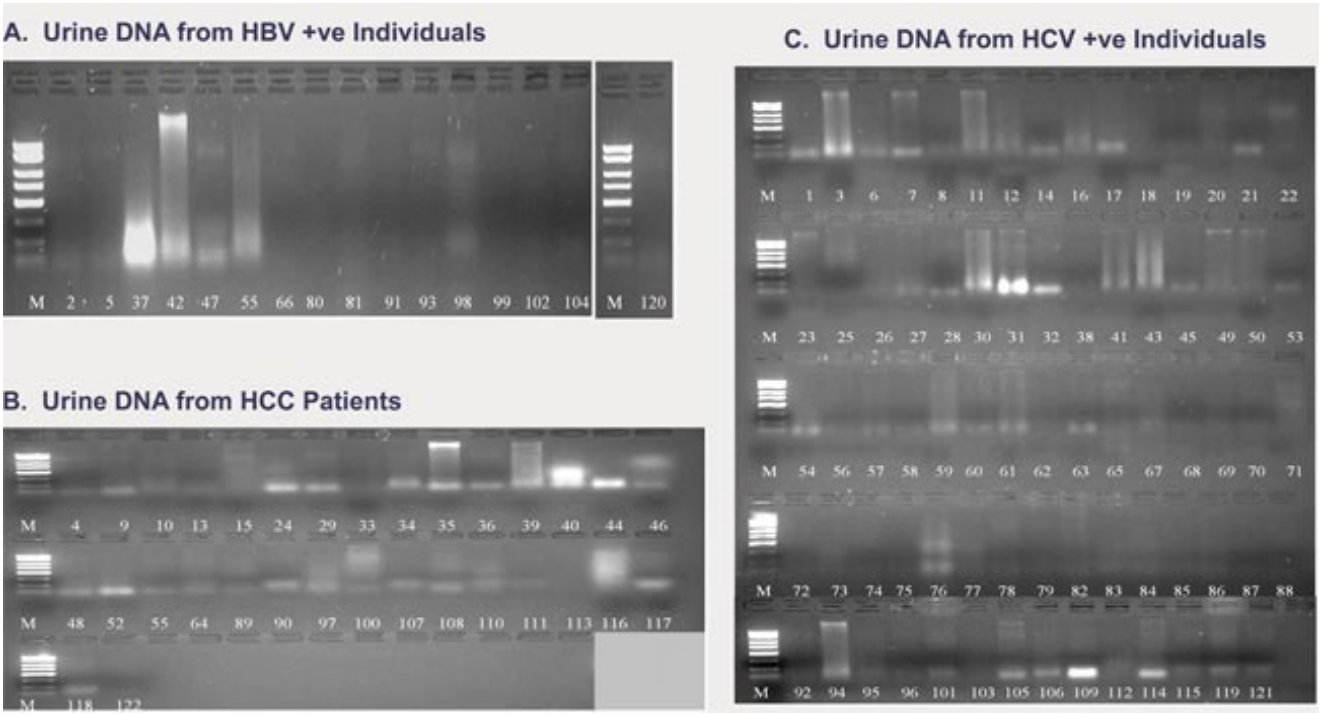

Figure 1: Agarose gels of DNA isolated using the Urine DNA Isolation Kit (Norgen Biotek) from 2 mL urine samples of individuals with HBV infections (Panel A), individuals with HCV infections (Panel C) and individuals with HCC post HCV infections (Panel B). A total of 20 µL out of each 100 µL elution was loaded onto a 2% agarose gel and run at 170 V for 25 minutes. M is the FastRunner DNA Ladder (Norgen Biotek). Pictures were taken using an AlphaImager™ 2200 from AlphaInnotech.

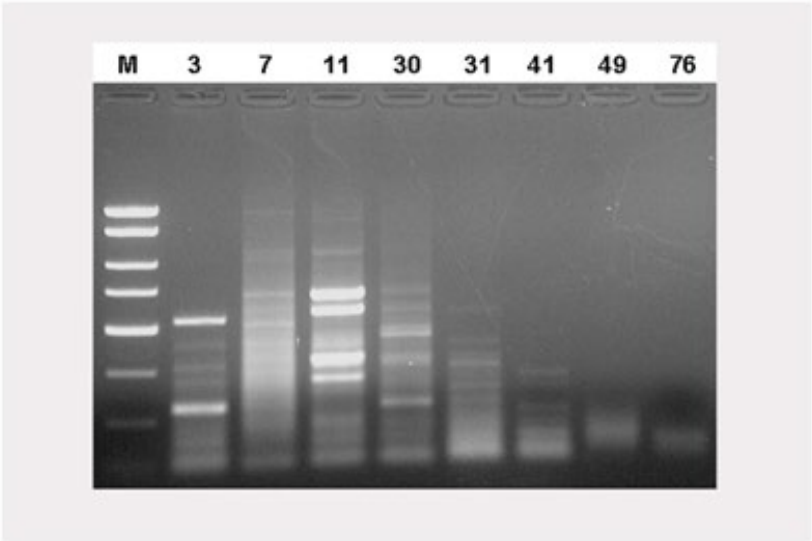

Figure 2: PCR amplification for the HCV 5' NCR gene from 8 different samples. The numbers above the lanes correspond to the sample number, and Lane M is Norgen's FastRunner DNA Ladder. The PCR product for the 5'NCR should be 256 bp, and this band appears in most of the samples. However, there is also the appearance of other non-specific products in most lanes. Sequencing of the 256 bp band showed that it is specific for the HCV 5'NCR. Sequencing was also performed on the non-specific bands, and a Blast search did not identify a specific HCV region but the sequence was found to be specific to the HCV viral genome.

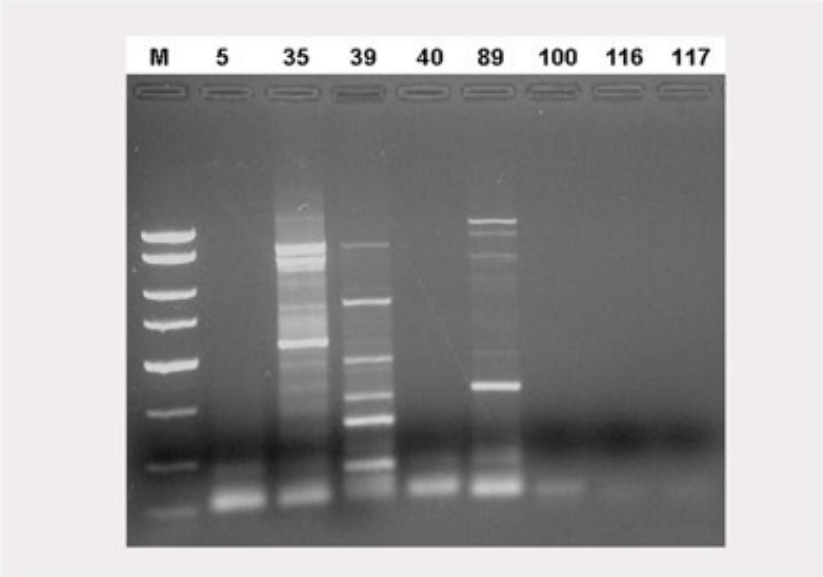

Figure 3: PCR amplification for the HBV polymerase and the surface genes from 8 different samples. The numbers above the lanes correspond to the sample number, and Lane M is Norgen's FastRunner DNA Ladder. Multiplex PCR for the polymerase and the surface genes was successful. Different band sizes which appear on the gel were confirmed by sequencing and Blast search to be of that for the HBV viral polymerase and the surface genes (preS2/preS1 genes). The reason for the different sizes of PCR products is because of the overlapping nature of these genes and also due to the possible different viral status of the viral genome during replication.

Conclusions

1. Genomic profiles from the HCC and HCV groups appeared to be similar. The HBV group's genomic profile differed in amount of gDNA present and the pattern of apoptotic DNA.
2. Based on genomic profiles it may be hypothesized that some of the patients in the HCV group may be progressing to HCC or they have already developed the cancer.
3. Amplification of HBV and HCV from the urine seems to be sensitive to the viral load.
4. Amplification of HCV 5' NCR was successful and analyzing of the product's sequence through HCV database showed that the PCR product is from serotype 4 which is predominant in Egypt.
5. Since the viral genome of the HBV is very complicated due to (1) the extensive overlapping of its genes, and (2) the viral DNA consists of incomplete dsDNA, it was very hard to amplify the certain genes directly form the isolated DNA.
6. Multiplex PCR amplification for the polymerase and the surface genes was successful but not in all the samples which needs more fine tuning.

References

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