Application of Next Generation Sequencing for Detection of Drug Resistance Mutations in Patients Infected with Chronic Viral Hepatitis B and C Diseases

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Introduction and Objectives

The hepatitis C virus (HCV) and hepatitis B virus (HBV) are blood-borne pathogens causing an inflammation of the liver that can lead to liver damage, hepatocellular carcinoma and death. Although most currently used treatment regimens for HCV and HBV infections can be initiated without prior knowledge of genotypes, however, success rates are negatively influenced by the high genetic variability represented by the circulating genotypes, and the drug-resistant variants among infected individuals specially those on long-term therapy. Although Sanger sequencing is the gold standard method for analysing viral mutations; thus, it is unable to determine the profile of a heterogeneous viral population in a patient. On the other hand, Next Generation Sequencing (NGS), is a high-throughput analysis platform which has been recently introduced into diagnostic virology laboratories. It has shown to be efficient in characterizing viral diversity and detecting minor mutants that constitute as little as 1% of the total viral population. Our objective was to optimize NGS for the detection of HBV and HCV genotypes and drug-resistance mutations especially low abundance variants circulating in Kuwait.

Methods

A total of 40 residual serum samples received for routine viral load testing from chronic hepatitis patients attending Gastroenterology Clinic at Mubarak Al Kabeer Hospital (20 for HBV and 20 for HCV) were used to optimize NGS methodology. NGS was standardized using ABL genotyping and drug-resistance assays that relies on specific-primers approach due to their high-resolution in identifying and classifying mixed and recombinant genotypes. The NGS workflow consisted of a robust methodology of the following: (i) for HCV, RT and nested-PCR amplification was applied for all HCV subtypes (1-6) based on NS3, NS5A and NS5BA genomic regions, **Figure 1**. (ii) for HBV, polymerase region was amplified, **Figure 2** in a one-round PCR to identify HBV genotypes and drug-resistance mutations of interest. Sequenced data were analysed using DeepCheck Analysis Software which subject raw data to a filtering process and phylogenetic classification using reference HCV and HBV genomes sequences provided from GenBank.

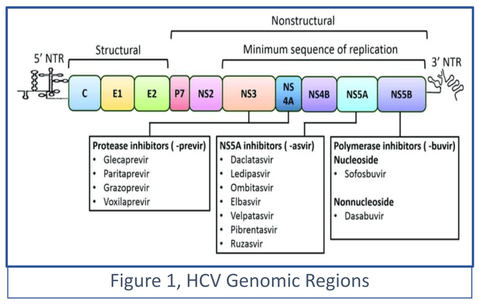

Figure 1, HCV Genomic Regions

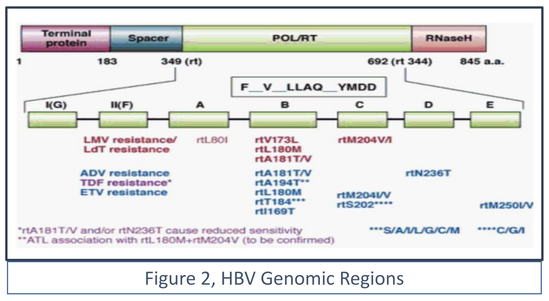

Figure 2, HBV Genomic Regions

Results and Discussion

All 40 serum samples (20 HCV and 20 HBV) were amplified as shown on **Figure 3** and **Figure 4**, and sequenced using Ion-Torrent S5 NGS platform (ThermoFisher). Sequencing was achieved with a genome coverage and depth of >90% of the HCV and HBV genomic regions covered by >100 reads/site.

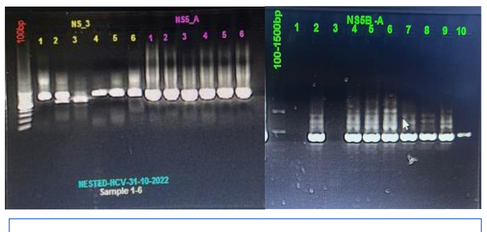

Figure 3. shows the amplification of NS3 (700 bp), NS5A (707 bp), NS5BA (693 bp) HCV genomic regions.

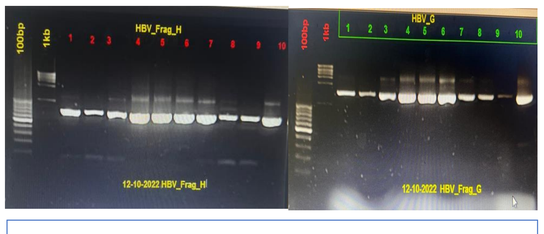

Figure 4. shows the amplification of *Pol* HBV genomic region, fragment G (1363 bp) and fragment H (760 bp).

Results and Discussion

Genotyping determination performed through homology testing of a consensus sequence generated from all the reads mapped to HCV and HBV genomic regions and compared to an updated set of reference sequences. Genotyping results for HCV and HBV (**Figure 5, A and B**) showed a prevalence of genotype 4a followed by 3a and less 1a in accordance with the previously published results on the predominant HCV genotypes in the Middle East. Similarly, prevalence of genotype D for HBV samples reported earlier to cause most severe liver disease.

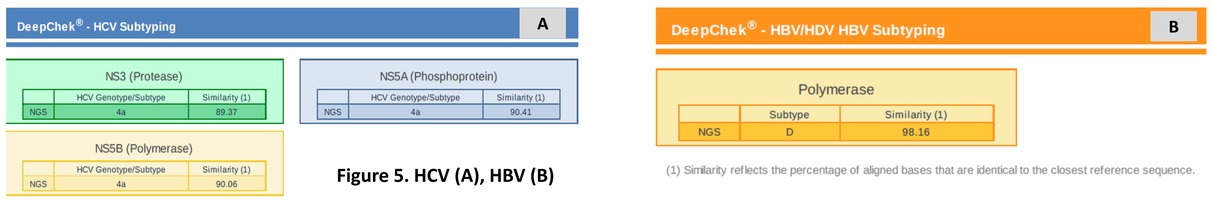

Figure 5. HCV (A), HBV (B)

Assessment of resistance-associated substitutions (RAS) and minority variants harboring RAS makes it possible to predict their selection with specific direct acting antivirals against HCV or HBV. This allows for the creation of a final report for RAS that includes recommendations for re-treatment of patients' refractory to the antiviral treatments. Accordingly, physicians will be provided with a summarized clinical report classifying the genotypes and drug-resistance mutations of interest along with treatment-regimen recommendations, **Figures 6 and 7**.

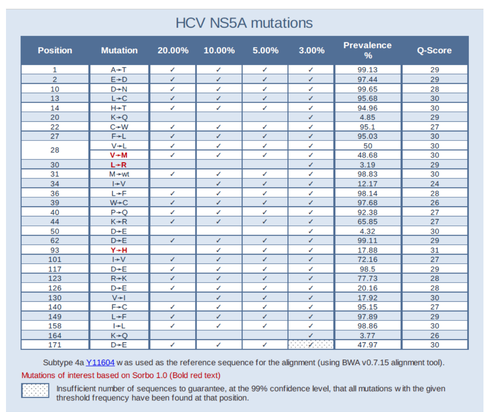

Figure 6. Resistant mutation Y93H and L30H were detected in (60%) of samples with HCV-4a and HCV-3a subtypes.

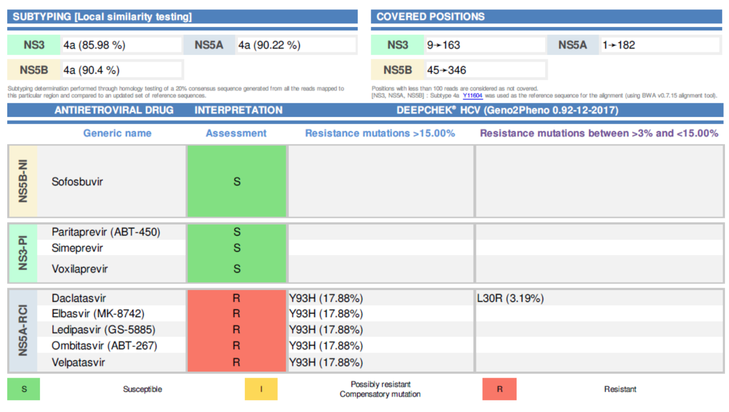

Figure 7. Detected mutations within NS5A region confer resistance to Daclatasvir, Elbasvir (MK-8742), Ledipasvir (GS-5885), Ombitasvir (ABT-267) and Velpatasvir.

HBV mutation detection revealed that all 18 samples showed susceptibility tendency to Adefovir, Entecavir, Lamivudine, Telbivudine and Tenofovir, while, two samples harboured mutation A181T that confer resistant to Adefovir, Lamivudine and Telbivudine **Figure 8** and **Figure 9**.

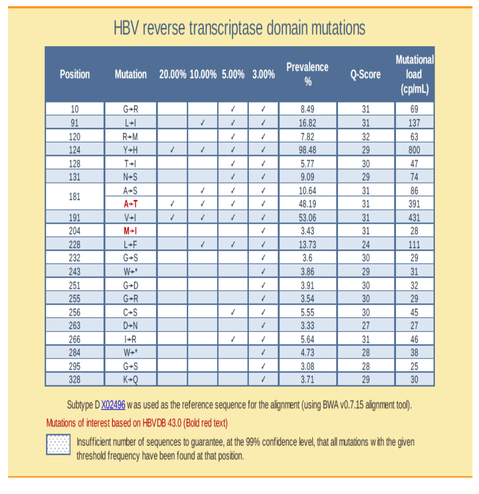

Figure 8. HBV NGS drug-resistant mutation panel.

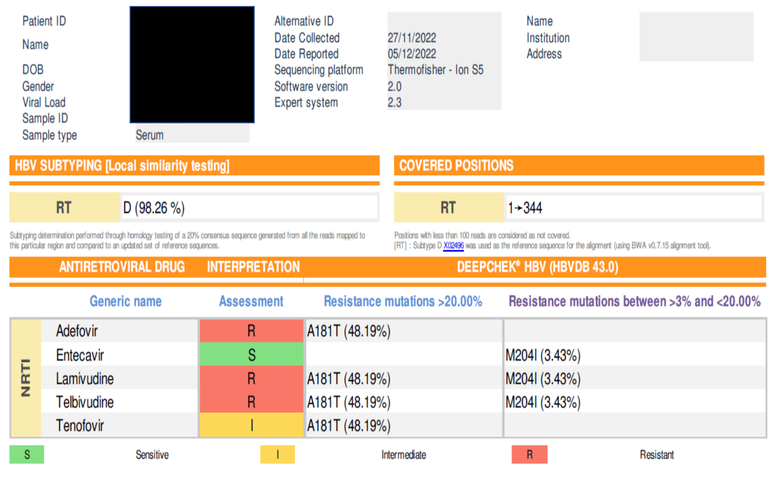

Figure 9. HBV NGS drug-resistant clinical report.

Conclusions: Due to common misclassifications by commercially available genotyping assays in combination with the limitations of currently used Sanger sequencing; this have raised the flag to investigate alternative diagnostic sequencing methods for the clinical management of patients infected with chronic viral HBV and HCV diseases. NGS technology can be helpful to physicians in guiding clinical practice and patient therapy management.

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