RESISTANCE ASSOCIATED VARIANTS TO NS5A AND NS5B INHIBITORS IN HEPATITIS C VIRUS OF NAÏVE CHRONICALLY INFECTED PATIENTS

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Introduction: Hepatitis C virus (HCV) is a major cause of chronic liver injury. The conventional pegylated interferon-ribavirin treatment, however, presents low sustained virological response (around 42%). Thus, new therapeutic options are under development; such as the direct acting antiviral non-structural (NS) protein inhibitors. Nevertheless, virus resistance associated variants (RAVs) have been frequently observed. Assessment of major and minor variant frequencies is important to identify emergent virus variants that can influence treatment outcome.

Objective: To identify RAVs in HCV NS5A and NS5B regions in patients chronically infected with HCV genotype 1a/1b.

Methods: Serum samples from 180 naïve or non-responders to previous treatment with pegylated interferon-ribavirin patients were collected. Viral RNA was extracted. Then, HCV NS5A and NS5B regions were amplified and sequenced. Sanger sequencing was used to screen for major viral population variants, while next generation sequencing (Ion PGM) was used to identify minor frequent variants. Geneious v.4.7.6 and CLC Genomics Workbench v.7.5 were used to identify variants by comparison to a reference sequence (AF009606 for HCV-1a and D90208 for HCV-1b).

Discussion and Results: Until now, HCV-NS5B region from 80 patients was screened by Sanger methodology. Four resistance mutations were found in HCV-NS5B region: A338V in 75% of patients infected with HCV-1a and 95% of patients infected with HCV-1b; C223Y in 12% and 10%, respectively; C316N and S368A in 47% and 7%, respectively, of patients infected with HCV-1a. Analysis of HCV-NS5A and NS5B from the others patients by both sequencing methodologies are under process.

Conclusions: We found resistant mutation variants to NS5B inhibitors in HCV-infected patients by Sanger sequencing. More detailed analysis with high throughput technology will be used to improve viral diversity characterization. Previous identification of RAVs can be useful for therapeutic management.

Key words: resistance mutations, direct acting antivirals, next generation sequencing