P-14 OPTIMIZATION OF MOLECULAR METHODS FOR SARS-CoV-2 QUALITATIVE DETECTION AND GENOTYPING IN RESPIRATORY SPECIMENS FROM PATIENTS WITH LIVER DISEASE

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Introduction and Objectives: SARS-CoV-2 active infection diagnosis is currently performed through RT-qPCR. Despite the fact that PCR-based assays can provide results relatively fast, these techniques require capable professionals, specific equipment and adequate infrastructure. In order to facilitate COVID-19 diagnosis in remote areas, an alternative to RT-qPCR would be loop-mediated isothermal (RT-LAMP) amplification. SARS-CoV-2 variant genotyping through high-throughput sequencing (HTS) allows SARS-CoV-2 genomic surveillance, especially for patients with a higher vulnerability. This study aimed to optimize RT-LAMP and HTS methods for SARS-CoV-2 RNA detection and genotyping, respectively, in respiratory samples from patients with liver disease.

Materials and Methods: A total of 142 respiratory secretions were obtained from individuals with SARS-CoV-2 RNA detectable by RT-qPCR (N1 Ct $\leq$ 30), divided into groups with (n=18) or without (n=124) liver disease. The study also enrolled 55 individuals who had SARS-CoV-2 RNA undetectable at RT-qPCR. For RT-LAMP methodology, primers were used for ORF1 gene amplification. As for HTS genotyping, the steps of cDNA synthesis, complete SARS-CoV-2 genome PCR amplification, preparation of genomic libraries and sequencing in MinION device were performed for 26 swab samples.

Results: Samples with viral RNA detectable by RT-qPCR had a mean Ct value of 24.3 $\pm$ 3.75. Referring to RT-LAMP, it was observed a sensitivity of 71.1% (101/142). When considering RT-qPCR mean Ct value, RT-LAMP sensitivity was 88.9% (16/18), associated with a mean Ct of 23.3 $\pm$ 3.5 for patients with COVID and hepatitis. A specificity of 100% (55/55) was observed since all negative swabs tested by RT-qPCR were negative at RT-LAMP. Through sequencing by MinION, SARS-CoV-2 lineages gamma (7/26; 27%), zeta (1/26; 3.9%), delta (6/26; 23%) and omicron (12/26; 46.1%) were genotyped and detected by RT-LAMP.

Conclusions: RT-LAMP demonstrated high sensitivity for molecular detection of SARS-CoV-2 RNA for patients with high viral load. Besides, RT-LAMP was capable of detecting all SARS-CoV-2 lineages genotyped by MinION in both groups.

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P-15 MMP-2 AND MMP-9 LEVELS IN ALCOHOLIC LIVER DISEASE, NON-ALCOHOLIC FATTY LIVER DISEASE AND CHRONIC HEPATITIS C

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Introduction and Objectives: It has already been reported that elevated serum levels were present in Hepatitis C patients, although they were found inactive. The behavior of gelatinases MMP-2 and -9 is yet unknown in other liver diseases. This study aimed to evaluate serum concentration of MMP-2 and -9 in different etiologies of liver disease also according to fibrosis stages.

Materials and Methods: Cross-sectional multicentric study, including subjects with no alcoholic fatty liver disease (NAFLD), chronic Hepatitis C (CHC), alcohol cirrhosis (CiOH) and alcoholism (OH), groups with alcohol drinking habits were classified according to WHO criteria, with clinical and biochemical evidence of alcoholic liver disease (ALD). Transient elastography (Fibroscan) was performed in NAFLD and CHC, considering mild fibrosis (FL: F0, F1, F2) and severe fibrosis (FA: F3, F4). As controls, subjects without alcohol consumption (CT) were recruited. Multiplex®-MERCK© was used for MMP-2 and -9 quantification. Statistical analysis was performed by Mann Whitney-U test, $p<0.05$, with SPSS V.22.

Results: The groups included were: 27 NAFLD (mild fibrosis: F0, F1, F2), 36 NAFLD (severe fibrosis: F3, F4), 48 CHC (mild fibrosis: F0, F1, F2), 54 CHC (severe fibrosis: F3, F4), 45 (CiOH), 99 (OH), and 138 CT. Both gelatinases, MMP-2 y MMP-9, were found elevated in CHC (mild and severe fibrosis) vs. CT; and decreased in OH, CiOH, HGNA (mild and severe fibrosis) vs. CT, plus there is significant differences between all etiologies, $p<0.001$.

Conclusions: In patients with CHC, MMP-2 y -9 serum concentration increases, particularly in severe fibrosis stages, although it has no effect on ECM (extracellular matrix) degradation, as they are inactive. Nevertheless, there is a significant decrease in these gelatinases in ALD and NAFLD. MMP-2 y MMP-9 are modulated according to etiological agents, which can be useful for the differential diagnosis of liver diseases.

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