ORT_22 - Multiplex real time pcr for molecular diagnosis of oncogenic viruses epstein-barr and human gammaherpesvirus 8 in hemodialyzed patients

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Introduction: The human gammaherpesviruses, in which Epstein-Barr virus (EBV) and Kaposi's sarcoma related herpesvirus (HHV-8), comprises viruses with a relevant oncogenic nature especially in immunodepressed hosts, such as hemodialyzed patients. In a coinfection, EBV and HHV8 have a synergetic effect on increased viral replication and tumorigenesis of associated cancers, worth mentioning Burkitt's lymphoma caused by EBV, primary effusion lymphoma (PEL) caused by both EBV and HHV-8, as well as Kaposi Sarcoma and Castleman's disease caused mainly by HHV-8. Therefore, it is fundamental simultaneous diagnosis of EBV and HHV-8 with high sensitivity and specificity in patients with high risk of developing associated neoplasms, such as hemodialyzed patients, aiming the EBV/HHV-8 viral load and tumorigenesis monitoring. Besides, saliva have shown to be the preferred biological sample used on this method, regarding practicality, low cost and less invasiveness during collection, the broad variety of biomarkers and viral presence available to be tested early on disease and less time consuming to extract target's genetic material.

Objectives: The aim of this study was to develop and optimize a multiplex assay protocol, based on the real time PCR technique, to detection of EBV and HHV8 in hemodialyzed patients.

Methodology: For this purpose, multiplex real-time PCR assays with synthetic standard curves, limit of detection (sensitivity test) and co-infection detection tests (specificity test) were performed. After real-time PCR optimization, 286 samples from hemodialyzed patients were tested for EBV/HHV-8 coinfection in saliva samples.

Results: The synthetic curves presented adequate parameters to be used, with values of slope= -3.406, R2= 0.999 and E= 96,6% for HHV8 and slope= -3,29, R2= 0.999 and E=101,35% for EBV. The limit of detection was set to 10^3 copies/mL for EBV and 10^3 copies/mL for HHV-8. The multiplex technique showed specificity >99%. Among the 286 samples tested, 32.5% (93/286) were EBV+, 2.8% (8/286) were HHV8+, and 4.5% (13/286) were co-infected with EBV+ and HHV8+.

Conclusion: The development of the multiplex qPCR protocol to detect simultaneously EBV and HHV8 has shown to be prominent and as specific and sensitive as the individual widely established protocols, presenting advantages in consuming less resources per assay and increasing diagnosis' speed, in addition to being less invasive with the use of saliva. Thus, it can be used with more vulnerable groups.

Keywords: Multiplex real time pcr, Epstein-Barr virus, Kaposi's sarcoma related herpesvirus