ID04. Rare-Variant-Sensitive High Resolution Melting Demonstrates that the His275Tyr Substitution in Pandemic H1N1 Influenza A Virus Appears after Oseltamivir Treatment in Infected Patients

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Introduction: Oseltamivir (Tamiflu) is widely used to treat pandemic 2009 (H1N1) influenza A. Since the start of the pandemic, 289 cases of oseltamivir resistance have been identified worldwide. All but one of these resistant viruses contain the 823C>T (His275Tyr) mutation in the neuraminidase gene. Although the burden of oseltamivir resistance is currently low, the threat of resistance is highlighted by the limitations of alternative therapies and the potential for rapid, global fixation of this mutation in the influenza A population. We developed an ultra-sensitive detection method to investigate when resistance emerges. **Methods:** Nine nasopharyngeal specimens from three pandemic 2009 (H1N1) influenza A patients were analyzed. Real-time PCR was used to monitor the presence of viral variants in patient samples. A Fluidigm BioMark™ digital array was used to quantitate neuraminidase gene copy numbers in test samples. To improve sensitivity of rare variant detection in patient samples, we developed a Rare-Variant-Sensitive High Resolution Melting (RVS-HRM) method to analyze the patient samples. **Results:** RVS-HRM detected the presence of very low copy numbers of individual alleles. In our sensitivity study, RVS-HRM was able to detect 0.5% of the resistant 275Tyr variant in the wild-type 275His neuraminidase gene. In addition, RVS-HRM detected the wild-type 275His allele among resistant 275Tyr variants in two patient samples in which real-time PCR detected only the resistant 275Tyr variant. Based on Poisson distribution, the probability of rare variant detection with 0.5% sensitivity is 95.65% in a 384-well plate. Real-time PCR detected wild-type pandemic 2009 (H1N1) influenza A virus in diagnostic specimens and the resistant 275Tyr strain after oseltamivir treatment from all three patients. RVS-HRM was used to investigate whether the original diagnostic samples contained very low amounts of 275Tyr. This much more sensitive method for rare variant detection demonstrated that, with a limit of detection of 0.5%, all diagnostic samples only contained the wild-type 275His allele. **Conclusions:** RVS-HRM is a highly sensitive technique that detects rare variants at a level of 0.5% with a 95.65% confidence level. Our analyses by both RVS-HRM and real-time PCR suggest that the resistant 275Tyr variant emerges only after oseltamivir treatment.