Expression of human toll-like receptor-2 in leptospirosis and improving the validity of gene expression analysis

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Introduction and Objectives: Identification of pathogen receptors by human immune cell receptors triggers the "immune responses". Toll-like receptors (TLRs) are the thoroughly studied immune cell receptors in mice models against leptospirosis. In this study, we aimed to improve the validity of gene expression analysis and to determine the response of the human TLR2 gene in confirmed patients for leptospirosis.

Methods: Clinically suspected patients were confirmed for leptospirosis using a previously validated qPCR. Total RNA was extracted from patients' whole blood followed by a DNase treatment. Messenger RNA(mRNA) specific exon-exon spanning primers (P-1) and conventional primers (P-2) targeting TLR2 gene were designed using NCBI tools, optimized for temperatures, validated by UCSC *in-silico* PCR and gel electrophoresis. Human TLR2 expression (n=10) was compared for the two primer sets and the gene expression analysis (n=18) was performed using P-1 on CFX Maestro™.GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was used as the housekeeping gene to calculate the gene expression value using ΔΔCq method.

Results: Of the 64 confirmed patients, 18 were selected with Cq(quantification cycle) value <37 and with ≥20 ng/µL of RNA quantity for the current study. Optimized annealing temperatures were 56℃ and 58℃ for P-1 and P-2 respectively. P-1 amplified only 1of 10 samples with a ΔΔCq of 0.01352, and it was identified as a ‘down-regulation’(dr). P-2 amplified the human TLR2 gene in 7 out of 10 (ΔΔCq=0.17891-dr, 0.70899-dr, 0.01415-dr, 0.00017-dr, 0.00390-dr, 0.67494-dr, 53.61714-up-regulation). Accordingly, P-1 was used in the gene expression study. In the analysis, 1 of 18 showed a 'dr' (ΔΔCq=0.01352) and the rest showed 'no change' of the TLR2 regulation (ΔΔCq=0.0000). The 'dr' of the TLR2 gene resulted with mean Cqof 29 and 38 respectively for GAPDH and TLR2. Of the17 resulted with 'no change', 12 didn't amplify the TLR2, but did amplify GAPDH with mean Cq values of 22,23,24,24,24,24,27,28,30,31,33,37. The rest of the 5 samples didn’t show gene amplification.

Conclusions: We confirmed the validity of exon-exon spanning primers in gene expression studies over the conventional primers. The reported up-regulationof TLR2 in mice models, wasn’t observed in this study population.

Keywords: Leptospirosis, Toll like receptos, Gene expression, Pathogenesis.

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