Accuracy of Reverse Transcription Loop-Mediated Isothermal Amplification Technique for Detecting Dengue Virus

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Abstract

In recent years, researchers have developed a reverse transcription loop-mediated isothermal amplification (RT-LAMP) test as a sensitive and specific technique. But the results of these studies were conflicting. The aim of this meta-analysis was to assess the accuracy of RT-LAMP technique for detecting dengue virus. We systematically searched PUBMED, EMBASE, Web of Science, and the Cochrane Library up to June 2015. Data from included studies were pooled to yield the summary sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary receiver operating characteristic (SROC) curve. All statistical analyses were performed using STATA VERSION 12.0 software. A total of 7 studies including 1263 clinical samples fulfilled the inclusion criteria. Our results showed that the pooled sensitivity and specificity were 0.99 and 0.96, respectively. The pooled DOR was 564.94 and the area under the curve (AUC) of SROC was 0.99, indicating a high level of overall accuracy. Besides, heterogeneity was statistically significant but was not caused by the threshold effect. Our study validates that RT-LAMP is an alternative molecular diagnostic method for the diagnosis of dengue virus infection.

Keywords: Dengue virus, reverse transcription loop-mediated isothermal amplification (RT-LAMP), accuracy.

INTRODUCTION

Dengue virus, a member of genus Flavivirus of the family Flavivirus, is transmitted by Aedes aegypti and Aedes albopictus mosquitoes (Kyle and Harris, 2008). There are four antigenically related but distinct serotypes of dengue virus
(dengue virus 1, 2, 3, and 4) (Russell and Nisalak, 1967), and each serotype contains several phylogenetically distinct genotypes (Holmes and Burch, 2000). The virus dengue virus infection induces a lifelong protective immunity to the homologous serotype, but it gives only a short time cross protective immunity against subsequent infection with any of the other three serotypes (Neeraja et al., 2015). Therefore, people may have multiple and sequential infections with the four dengue virus serotypes in a region where the infection is hyper endemic due to the lack of cross-protective neutralizing antibodies (Neeraja et al., 2015).

It is estimated that 390 million dengue virus infections are believed to occur each year, of which 96 million are clinically symptomatic (Bhatt et al., 2013). Prior dengue virus infection is a major risk factor for the subsequent development of fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) through antibody-dependent enhancement (Maves et al., 2010). Timely and accurate diagnosis of dengue virus infection can reduce the number of cases of DHF and DSS. Obviously, diagnosing dengue virus infection is vitally important for guiding appropriate supportive care and for preventing potential dengue outbreak. However, dengue fever presents clinical characteristics similar to other febrile illness (Ferraz et al., 2013). Therefore, a laboratory method for the rapid and accurate diagnosis of early dengue virus infection is highly needed.

Molecular techniques to detect virus genomic RNA sequence by the reverse transcription polymerase chain reaction (RT-PCR) and the real-time quantitative (qRT-PCR) have been accepted as the new standard method for the detection of dengue virus (Melo et al., 2006), which enable diagnosis during the acute phase of dengue virus infection (Lanciotti et al., 1992; Shu et al., 2003). However, these PCR-based methods require either high-precision instruments for the amplification or elaborate methods for detection of the amplified products (Neeraja et al., 2015; Parida et al., 2007). In recent years, reverse transcription loop-mediated isothermal amplification (RT-LAMP) as a novel nucleic acid amplification method has the potential to replace the RT-PCR owing to its rapidity, sensitivity, specificity and cost-effectiveness without the need of specialized equipment (Tomita et al., 2008).

To facilitate needed diagnosis, we performed a systematic review and meta-analysis to investigate the performance of RT-LAMP assay for diagnosis of dengue virus infection when compared with RT-PCR or qRT-PCR.

MATERIALS AND METHODS

This systematic literature review was performed according to the PRISMA Statement (Moher et al., 2009) and Cochrane Collaboration guidelines (http://handbook.cochrane.org/). Study validity was assessed on the basis of the Standards for Reporting of Diagnostic Accuracy Initiative and the Review of Methodological Standards (Bossuyt et al., 2003).

Search strategy

We conducted a systematical search of the PUBMED, EMBASE, Web of Science, and the Cochrane Library using the following terms: (“Dengue Virus” OR “Dengue Viruses” OR “Breakbone Fever Virus” OR “Breakbone Fever Viruses”) AND (“loop-mediated isothermal amplification method” OR “RT-LAMP” OR “LAMP”) AND (“diagnosis” OR “detection” OR “accuracy” OR “screening” OR “sensitivity” OR “specificity”). No language or publication date restrictions were applied to the search. This search was performed in June 2015. Searching was conducted independently by two reviewers (Guo-Ming Su and Wei-Xi Yuan), and discrepancies were resolved by consensus opinion. To ensure comprehensive
acquisition of literature, the reference lists of retrieved studies were scanned to identify additional relevant article.

**Inclusion and exclusion criteria**

Studies evaluating RT-LAMP as a diagnostic test for dengue virus infection were eligible for inclusion if the studies 1) used RT-PCR or qRT-PCR as the reference standard; 2) performed clinical samples analyses from patients clinically suspected with dengue virus infection; 3) contained no less than ten specimens; 4) provided sufficient data that allowed calculation of true positive (TP), false positive (FP), false negative (FN), and true negative (TN). Relevant studies were excluded if they were review articles, meta-analysis, opinions, editorials, commentaries, or conference abstracts. Two reviewers (Guo-Ming Su and Chun-Cai Hu) independently screened studies according to eligibility criteria. Any discrepancies were resolved by consensus or by correspondence with study authors.

**Data extraction**

Two reviewers (Guo-Ming Su and Wei-Xi Yuan) independently extracted information from the selected papers, and then another reviewer verified them (Zu-Guo Zhao). Disagreements between reviewers were resolved by discussion with the involvement of an arbitrator if necessary. The following information was collected from each study: the first author, publication year, study location, reference standard, sample size, and data for two by two tables (TP, FP, FN, and TN), respectively. For each study, these were summarized as sensitivity = TP / (TP + FN) × 100%; specificity = TN / (TN + FP) × 100%; positive predictive value (PPV) = TP / (TP + FP) × 100%; negative predictive value (NPV) = TN / (TN + FN) × 100%; and prevalence = (TP + FN) / (TP + FN + TN + FP) × 100%.

**Data analysis**

To determine the diagnostic accuracy of RT-LAMP, correlated diagnostic accuracy indexes were computed as follows: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic odds ratio (DOR) along with 95% confidence intervals (95% CI). The PLR represents the value by which the odds of the disease increase when a test is positive; whereas NLR shows the value by which the odds of the disease decrease when a test is negative. The DOR reflects the relationship between the result of the diagnostic test and the disease, the value of which ranges from 0 to infinity-higher values indicating better discriminatory test performance (Glas et al., 2003). Summary receiver operating characteristic (SROC) curve was also used to summarize overall test performance (Rosman and Korsten, 2007). The area under the curve (AUC) under the SROC curve is a measure of the overall performance of a diagnostic test to accurately differentiate those with and those without the condition of interest (Walter, 2002).

Heterogeneity was assessed using the Cochran Q chi-square test and the $I^2$ statistic (Higgins and Thompson, 2002; Huedo-Medina et al., 2006). If there was no significant heterogeneity ($p$-value > 0.05 and $I^2 < 50$%) among studies, the fixed-effect model (Hedges, 1998) was performed for the meta-analysis; otherwise, the random-effect model (Schmidt, Oh, and Hayes, 2009) was chosen. To address potential heterogeneity among studies, we also performed a subgroup analysis based on reference standard. Spearman model was then applied to explore the threshold effect on the
performance of the RT-LAMP assay. Fagan's nomogram, a two-dimensional graphical tool, was used to estimate how much the result of a diagnostic test changes the probability that a patient has a disease (Fagan, 1975). Publication bias was detected using Deeks' regression test of asymmetry (Deeks, Macaskill, and Irwig, 2005). All analyses were undertaken using STATA VERSION 12.0 software (StataCorp, 2011).

RESULTS

Search results

A total of 74 titles and abstracts were retrieved after the primary search of the electronic databases for published work on the subject. Sixty-eight potentially relevant citations were selected based on relevance to the study topic. After reviewing the titles and abstracts, 57 citations were excluded as basic science studies, or detecting different viruses. Then 11 articles were selected for full-text review. Subsequently, 4 studies were excluded (Dauner et al., 2010; Kwallah et al., 2013; Li et al., 2011; Teoh et al., 2013a) (reasons for exclusion in Figure 1). Finally, seven articles reported the sensitivity and specificity of RT-LAMP on clinical samples for the diagnosis of dengue virus infection and were selected in our data analysis (Dauner et al., 2015; Lu et al., 2012; Neeraja et al., 2015; Parida et al., 2005; Sahni et al., 2013; Teoh et al., 2015;
Figure 2. Forest plot of the sensitivity (left) and specificity (right) for studies using RT-LAMP assay to detect dengue virus infection. The sensitivity and specificity are represented by individual squares, and the horizontal lines represent the 95% CIs for each included study. The diamonds represent the pooled summary estimates (95% CI).

Teoh et al., 2013b). The details of study selection flow are summarized in Figure 1.

Study characteristics

Our systematic review included 7 studies, 43% of which were published in 2015. Among the studies included in the meta-analysis, there were a total of 1263 clinical samples. All samples (serum or plasma) were from patients suspected dengue virus infection. As for reference standards, five studies used RT-PCR assay (Dauner et al., 2015; Lu et al., 2012; Neeraja et al., 2015; Parida et al., 2005; Sahni et al., 2013), and two used qRT-PCR assay (Teoh et al., 2015; Teoh et al., 2013b). Prevalence of dengue virus infection in each study was highly variable, ranging from 26.2% to 77.1%. The main characteristics of the included studies are shown in Table 1.

Overall diagnostic performance of RT-LAMP

Using bivariate mixed-effects models, the combined results were as follows: sensitivity was 0.99 (95% CI, 0.84 - 1.00); specificity was 0.96 (95% CI, 0.90 - 0.99); PLR was 27.3 (95% CI, 9.2 - 80.4); NLR was 0.01 (95% CI, 0.00 - 0.19). The forest plot of sensitivity and specificity for RT-LAMP method in the detection of dengue virus infection was shown in Figure 2. The NLR was 0.01, which suggested that if a RT-LAMP result was negative, the probability rate of the individual
Figure 3. Overall diagnostic odds ratio (DOR) for all data sets describing the diagnostic performance of RT-LAMP in detecting dengue virus infection.

having dengue virus infection was 1% in theory. In contrast, the PLR value was 27.3, suggesting that patients with a positive RT-LAMP result had a about 27 fold chance of being diagnosed with dengue virus infection. Using random effects analysis, the pooled DOR was 564.94 (95% CI, 127.29 – 2507.43) (Figure 3), with individual DORs ranging from 83.92 to 5041.47. As was shown in Figure 4, the AUC of SROC curve based on summary sensitivity and specificity across all data sets were 0.99 (95% CI, 0.98 - 1.00), indicating a high level of overall accuracy.

Subgroup analysis by RT-PCR

We performed a subgroup analysis in five studies, which used RT-PCR as a reference standard. The pooled sensitivity, specificity, PLR, NLR, and DOR were 1.00 (95% CI, 0.36 - 1.00), 0.94 (95% CI, 0.82 - 0.98), 17.3 (95% CI, 5.1 - 58.7), 0.00 (95% CI, 0.00 - 1.85), and 746.58 (95% CI, 102.57 - 5434.16), respectively. The SROC curve indicated that the area under the curve (AUC) was 0.99 (95% CI, 0.98 - 1.00). The result of subgroup analysis also displayed a good diagnostic accuracy.

Investigation of heterogeneity

We used the Cochran Q chi-square test and the I² statistic to evaluate the presence of statistical heterogeneity.
Significant heterogeneity was found for the pooled sensitivity, specificity, PLR, NLR, and DOR. So we performed an analysis of diagnostic threshold to explore the effect on the performance of the RT-LAMP assay. Spearman correlation coefficient was found to be 0.286 with a \( p \) value of 0.535, indicating that there was no statistically significant difference.

**Posttest probability**

The relationship between pretest probability and posttest probability was depicted by visual Fagan’s nomogram (Figure 5). We performed a simulation of an environment that had a prevalence of 20% for dengue virus infection, with base on the included studies. As a result, the probability in this model of someone having the disease and not being detected by the RT-LAMP test was 0.3%. In contrast, the posttest probability of sick patients with a positive test was 87%.

**Publication bias**

Deeks’ funnel plot asymmetry test indicated that no significant bias was found (\( t = -0.83; p = 0.45 \)). The shape of the
Figure 5. Fagan’s nomogram for the calculation of posttest probability.
Table 1. Characteristics of the included studies.

<table>
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<tr>
<th>First author</th>
<th>Year</th>
<th>Region</th>
<th>Reference standard</th>
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<th>TP</th>
<th>FP</th>
<th>FN</th>
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<th>PPV</th>
<th>NPV</th>
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<tr>
<td>B.T. Teoh</td>
<td>2015</td>
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<td>qRT-PCR</td>
<td>203</td>
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<td>5</td>
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<td>90.50%</td>
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RT-PCR, reverse transcription polymerase chain reaction; TP, true-positive; FP, false-positive; FN, false-negative; TN, true-negative; NPV, negative predictive value; PPV, positive predictive value.

funnel plot also did not show any evidence of obvious asymmetry (data not shown), suggesting that there was no potential publication bias.

DISCUSSION

Dengue virus infection is a major public health problem causing serious morbidity and mortality in tropical and subtropical developing countries (Sahni et al., 2013; Tavakoli et al., 2007). An excellent method for the rapid diagnosis of dengue virus infection is highly needed. Regarding limitations of traditional techniques for detection of dengue virus, researchers have developed a reverse transcription loop-mediated isothermal amplification (RT-LAMP) test as a sensitive and specific technique (Dauner et al., 2010; Parida et al., 2005; Sahni et al., 2013). However, the results of these studies showed that the sensitivity of RT-LAMP assay ranged from 75.4% to 100% (Neeraja et al., 2015; Sahni et al., 2013; Teoh et al., 2015), and that the specificity ranged from 73.4% to 100% (Lu et al., 2012; Parida et al., 2005; Sahni et al., 2013). Hence, a systematic review and meta-analysis that assessed the accuracy of RT-LAMP technique for detecting dengue virus was highly needed.

The RT-LAMP assay is a novel method of gene amplification, which is based on the principle of a strand displacement reaction and stem-loop structure that amplifies the target with high degrees of specificity and selectivity and with rapidity under isothermal conditions (Nagamine, Hase, and Notomi, 2002; Notomi et al., 2000). As a matter of fact, the RT-LAMP test has recently emerged as a powerful gene amplification tool for rapid identification of multiple viruses, such as the rubella viruses (Abo et al., 2014), hepatitis D virus (Wang et al., 2013), and bovine rotavirus (Xie et al., 2012).

In our study, the RT-LAMP assay demonstrated high sensitivity and specificity in comparison to RT-PCR or qRT-PCR as the reference standard for diagnosing dengue virus infection. Despite statistical heterogeneity, the diagnostic accuracy was consistently similar in the direction of effect in the majority of the studies. To illustrate the overall performance of
RT-LAMP test, we also counted the AUC of the SROC curve. In present meta-analysis, the data showed that the pooled DOR was 564.94, suggesting a high level of overall accuracy. In addition, our result showed that the AUC was 0.99, also indicating very good ability to diagnose dengue virus infection. However, compared with the SROC curve and DOR, the likelihood ratio is considered to be more clinically meaningful for our measures of diagnostic accuracy (Gallagher, 1998). The likelihood ratios for the RT-LAMP assay indicated that the test is useful in determining posttest probability of dengue virus infection.

To explore sources of heterogeneity, we used the Spearman correlation coefficient to analyze the threshold effect. The result suggested that the heterogeneity was not caused by the threshold effect. However, statistically significant heterogeneity was observed when we pooled sensitivity, specificity and DOR of included studies, implying that there should be other factors rather than threshold effect resulting in variations among studies. Perhaps heterogeneity was caused by the conditions of RT-LAMP such as nucleic acid isolation and primer design. But it was overwhelmingly difficult to compare the success of each RT-LAMP condition due to diversity. To address potential heterogeneity among studies, we performed a subgroup analysis based on RT-PCR. Results remained robust after subgroup analysis.

However, two limitations should be acknowledged in our study. On one hand, sample size after pooling the existing studies was still relatively small. This meta-analysis only selected seven studies, though we performed a systematical search of main electronic databases. On the other hand, there was substantial heterogeneity for all the statistical measures. Although an effort was made, we failed to find a major source of heterogeneity due to insufficient data.

In conclusion, although several limitations exist, our findings suggest that RT-LAMP is a useful diagnostic tool with a high sensitivity, specificity, likelihood ratios, and posttest probability in the detection of dengue virus. However, to better investigate the possible sources of between-study heterogeneity, further systematic review involving more studies with meta-regression analysis should be performed in the future.

ABBREVIATIONS

CI, confidence interval; FN, false-negative; FP, false-positive; TN, true-negative; TP, true-positive; RT-LAMP, reversed transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; DOR, diagnostic odds ratio; PLR, positive likelihood ratio; NLR, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; summary receiver operating curve; AUC, area under the curve.

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