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PERFORMANCE OF RAPID ANTIBODY TEST AND RT-PCR AS FRONTLINE TEST FOR COVID-19 DIAGNOSIS IN PREGNANCY: AN EXPERIENCE IN INDONESIA

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Abstract. Ensuring an accurate diagnosis is critical for limiting the spread of SARS-CoV-2 and for the clinical management of COVID-19, especially in pregnant women. For now real-time reverse transcription polymerase chain reaction (RT-qPCR) is the currently recommended laboratory method for the diagnosis of acute SARS-CoV-2 infection. More recently, several easy-to-perform rapid antigen detection tests have been developed and are recommended as first-line screening test in several countries. The purpose of this study was to evaluate the comparative performance of a rapid antibody test and RT-PCR for the detection of SARS-CoV-2 infection, as a front-line test for the diagnosis of COVID-19 in pregnancy. This research method is a descriptive study to describe comparation of sensitivity and specificity between rapid SARS-CoV-2 antibody test to the gold standard nasopharyngeal RT-PCR swab test. Of the 271 samples, only 257 were eligible and fourteen cases were excluded from the study due to a lack of rapid antibody test and RT-PCR results. The results of this study showed that the rapid SARS-CoV-2 antibody test sensitivity was 80.95%, and the specificity was 90.68%, the NPV (negative predictive value) and the PPV (positive prognosis value) were 98.17% and 43.59%, respectively. Based only on the results of IgM and IgG, IgM and IgG sensitivity were 33.33% (7/21) and 71.43% (15/21), respectively, and the specificity was 91.1% (215/236, 21 false positive) and 91.53% (216/236, 20 false positive), respectively. The use of rapid antibody tests during pregnancy is a screening tool and is not currently applicable for diagnostic tool. To minimize false positives and negatives results, the use of rapid antibody tests should be combined with the RT-PCR test results.

Keywords: COVID-19; rapid antibody detection test; SARS-CoV-2; RT-PCR; pregnancy.

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INTRODUCTION

The COVID-19 virus is spreading rapidly from person to person in China, and the World Health Organization (WHO) has reported an outbreak of COVID-19 is now spreading globally (Almaghaslah et al., 2020). Highly sensitive and specific tests are critical for the diagnosis and treatment of COVID-19 patients (Scohy et al., 2020)

This was announced by the Centers for Disease Control and Prevention (CDC), pregnant women appear to be at the same risk as non-pregnant adults, with data on pregnancy status available for 91,412 (28.0%) women with laboratory-confirmed infection; of these, 8,207 (9.0%) were pregnant (Berghella, et al., 2020). The reliable laboratory testing is necessary because the number of suspected cases increases. RT-PCR testing of asymptomatic or mildly symptomatic individuals may be considered when evaluating people who have been in close contact with a confirmed case of COVID-19 (Scohy et al., 2020); WHO, 2020).

Real-time reverse transcription polymerase chain reaction (RT-qPCR) is the currently recommended method for the diagnosis of acute SARS-CoV-2 infection. However, several factors such as specialized equipment and skilled personnel limit the use of this method (Scohy, et al., 2020).

Higher viral load linked to better antigen detection rates and antibody formation in blood (Scohy, et al., 2020; WHO, 2020). Seroconversion may not occur 1-3 weeks after onset of the symptoms, so this test method may be of limited use in diagnosing acute infections. However, detection of anti-SARS-CoV-2 antibodies in serum can be used to determine the transmission chains, which may be useful for contact tracing investigation (Berghella, et al., 2020).

Currently, all government and private hospitals in Bali use rapid antibody tests as to screening all pregnant women who are going to have obstetric procedure. However, many of the cases detected using the SARS-CoV-2 rapid antibody test had negative swab results. In this study, we present the results of two diagnostic methods: a serum total antibody assay against SARS-COV-2 and RT-PCR for detection of SARS-COV-2 infection in pregnant women.

The purpose of this study was to compare the performance of a rapid antibody test as a front-line test and RT-PCR for the diagnosis of COVID-19 in pregnancy.

METHODS

This research method is a descriptive study to describe comparation of sensitivity and specificity between rapid SARS-CoV-2 antibody test to the gold standard nasopharyngeal RT-PCR swab test.

Test

The COVID-19 RT-PCR test is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test for the qualitative detection of SARS-CoV-2 nucleic acid in upper and lower respiratory tract samples (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirate, bronchoalveolar lavage and nasopharyngeal lavage/ aspirate). Laboratory diagnosis of COVID-

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19 at Udayana Hospital and Sanglah Hospital relies on RNA extracts to detect viral RNA by targeting the RNA-dependent RNA polymerase (RdRp) gene (De Kauwe et al., 2020). Amplification was performed on a LightCycler 480 instrument (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. Samples with a SARS-CoV-2 RT-qPCR cycle threshold (Ct) below 40 were considered positive.

The Autobio Anti-SARS-CoV-2 Rapid Test is based on a one-step detection method. The cassette contains membranes pre-coated with two mouse anti-human monoclonal antibodies (anti-IgG and anti-IgM) on two separate assay lines. SARS-CoV-2 recombinant spike protein antigen reagents capable of specific binding to SARS-CoV-2 antibodies (IgM and/or IgG) were bound to colloidal gold and sprayed onto the conjugate pad. When the sample is applied to the test well, a complex of antibody and labeled antigen are formed and migrates to the top of the strip. Gold-labeled colorimetric reagents are used to form visible red/pink lines.

Statistics

This research used sensitivity and specificity as criteria to assess the performance of SARS-CoV-2 rapid antibody test. RT-PCR is considered the gold standard for this evaluation, so positive and negative samples detected by RT-PCR are considered true positives and true negatives.

RESULTS AND DISCUSSION

We collected 271 pregnancy samples from referral hospitals (Udayana University Hospital and Sanglah Hospital) from March 2020 to April 2020, of all cases, only 257 samples were eligible, 14 samples were excluded from this study due to lack of rapid SARS-CoV-2 antibody tests and RT-PCR test results

Table 1. Distribution of study population

Variable		f	%
Age	>35 year old	45	17.5
	≤ 35 year old	212	82.5
	Total	257	100
Gravida	1	80	31.1
	2	85	33.1
	3	54	21
	4	23	8.9
	5	11	4.3
	6	3	1.2
	7	1	0.4
	Total	257	100
Parity	0	100	38.9
	1	82	31.9
	2	53	20.6
	3	13	5.1
	4	9	3.5
	Total	257	100
Trimester	1st trimester	1	0.4
	2nd trimester	6	2.3
	3rd trimester	250	97.3
	Total	257	100
Referral	Referral cases	38	14.8
	Not referral cases	219	85.2
	Total	257	100
Rapid test reactive	yes	49	19.1
	No	208	80.9
	Total	257	100
IgM(+),IgG(+)	yes	5	1.9
	No	252	98.1
	Total	257	100
IgM(+),IgG(-)	yes	13	5.1
	No	244	94.9
	Total	257	100
IgM(-),IgG(+)	yes	21	8.2
	No	236	91.8
	Total	257	100

Variable		f	%
IgM(-),IgG(-)	yes	4	1.6
	No	253	98.4
	Total	257	100
RT-PCR	positive	21	8.2
	negative	236	91.8
	Total	257	100
Obstetric Management	CS	134	52.2
	Vaginal delivery	116	45.1
	Conservative management	6	2.3
	Curettage	1	0.4
	Total	257	100
Symptoms	yes	5	1.9
	No	252	98.1
	Total	257	100

The above data shows that 82.5% (212/257) of pregnant women are under 35 years old and 17.5% (45/257) are over 35 years old and most of the samples and controls are in her second pregnancy 33.1% (85/257). Most cases are admitted to the hospital in the third trimester (97.3%, 250/257), 85.2% cases (219/257) were non-referral cases, and most obstetric management done in this study was caesarean section which performed in 52.2% cases (134/257), 116 cases (45.1%) had vaginal delivery, only 6 cases were managed conservatively until the RT-PCR swab test result was negative, and 1 case had an incomplete abortion and curettage procedure has been carried out. Of all the cases and controls, only 5 cases (1.9%) had clinical symptoms and 252 cases (98.1%) were asymptomatic.

Table 2. The sensitivity and specificity of SARS-CoV-2 IgG-IgM combined antibody to the detection of SARS-CoV-2 infection in pregnant women

	Clinical Positive sample	Clinical Negative sample
Sample Quality	21	236
IgM-IgG reactive	3	2
IgM reactive	3	10
IgG reactive	11	10
Sensitivity	80,95%	
Specificity		90,68%

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Blood samples were taken from COVID-19 patients at Udayana University Hospital and Sanglah Hospital and the data results were collected from the Clinical Pathology laboratory of these two hospitals. A total of 257 cases were tested: 21 cases were confirmed COVID-19 clinically and had positive RT-PCR results, while the other 236 cases were discarded from COVID-19 and had negative RT-PCR results. Due to time constraints, we did not have detailed data for how long each patient has been infected or how long they had symptoms when the blood samples were collected at Udayana University Hospital and Sanglah Hospital. Of all the data above, the reactive results of rapid SARS-CoV-2 antibody test has 80.95% sensitivity and the specificity was 90.68%, the NPV (negative predictive value) and PPV (positive predictive value) were 98.17% and 43.59%, respectively.

Table 3. Comparisons of IgM and IgG results for 21 cases with RT-PCR positive COVID-19 cases and 236 cases with discarded COVID-19.

		RT-PCR	
		Positive	Negative
IgM	Positive	7	21
	Negative	14	215
IgG	Positive	15	20
	Negative	6	216

Based on the above results, the study showed a sensitivity of 33.33% (7/21) and 71.43% (15/21) for IgM and IgG, respectively. The IgM and IgG overall specificity was 91.1% (215/236, 21 false positive) and 91.53% (216/236, 20 false positives), respectively.

There are three types of tests for diagnosing viral infections: Reverse Transcription Quantitative Polymerase Chain Reaction (RT-PCR), viral antigen detection tests and serological immunoassays that detect human response to virus-specific antibodies (IgM and IgG), as of serological tests based on antibodies could be very helpful (Kontou, Braliou, Dimou, Nikolopoulos, & Bagos, 2020).

Overall evidence for the SARS-CoV-2 IgG/ IgM assay reported its sensitivity was 88.66% and its specificity was 90.63% (Li et al., 2020). Using RT-PCR confirmed case as true positive, the accuracy if the test was 94.1% (144/153) for IgM and 98.0% (150/153) for IgG (Hoffman et al., 2020). The combined IgG-IgM antibody test kit has 88.66% sensitivity and 90.63 specificity in his study found a sensitivity of 92.2 %, 95.7 % and 98.6 % for the RT-PCR, the total antibody test (Li et al., 2020), and the combined method, respectively, and the specificity are 100 %, 98.7 %, and 98.7 %, respectively (Pei Wang, 2020).

This study showed that the SARS-CoV-2 antibody rapid test has a sensitivity of 80.95%, a specificity of 90.68%, and a NPV and PPV of 98.17% and 43.59%, respectively, when compared with RT-PCR results. When we compared each IgM and IgG individually, the sensitivity and specificity of IgM were 33.33% (7/21) and

91.1% (215/236), respectively, and the sensitivity and specificity of IgG were 71.43% (15/21) and 91.53% (216/236), respectively. The false negative results may be mainly due to a low concentration of antibodies, so the test result will be negative. Second, differences in antibody production of individual immune responses may be responsible for false-negative results in COVID-19 patients. Third, IgM antibodies will be disappear after 2 weeks (Li et al., 2020).

This new combined SARS-CoV-2 IgG-IgM antibody test kit has several benefits. Compared to RT-PCR, it is more time saving, requires no equipment, is easier to do, and requires minimal training. Serology antibody testing is essential in patients with mild to moderate disease who may present later (2 weeks after symptoms onset). Serological diagnosis is also important for tracing COVID-19 contact in community and for identify immunity status of the individuals, whether they already had "protection" from SARS-CoV-2 infection or not (Sethuraman, Jeremiah, & Ryo, 2020).

Although it is the most sensitive test for diagnosing COVID-19, RT-PCR still has its limitations. RT-PCR accuracy requires high-quality nasopharyngeal swab that contain sufficient viral RNA and transport medium and extraction steps. This can vary even in the same patient, depends on the time of the test, the onset of infection and/ or the onset of symptoms. In the absence of sufficient viral RNA, RT-PCR may provides false negative test results (Li et al., 2020). The RNA detection results depend on sample quality, extracted RNA, RT-PCR reagents source, and multiple steps in RNA preparation. In addition, different sample types gave different positive identification rates ranging from 1% to 93% (Wenling Wang et al., 2020).

One study showed that RT-PCR had high specificity (100%) but relatively low sensitivity (92.2%) (Pei Wang, 2020). This is due to its primary design is spesific for the SARS-CoV-2 genome sequences. Occasional false positives results can occur due to technical errors and reagent contamination (Sethuraman et al., 2020).

The seventh edition guideline for COVID-19 issued by the National Health Commision of the People's Republic of China recommends serology testing as a supporting proof for COVID-19, however virus RNA detection by RT-PCR method has become the standard diagnostic test for confirming SARS-CoV-2 infection (Pei Wang, 2020).

CONCLUSIONS

Based on the above results, this study showed that IgM and IgG has a sensitivity of 33.33% and 71.43%, respectively, and a specificity of 91.1% and 91.53%, respectively.

The use of rapid antibody tests during pregnancy is a screening tool and is not currently applicable for diagnostic tool. To minimize false positives and negatives results, the use of rapid antibody tests should be combined with the RT-PCR test results.

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