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Postmortem diagnosis characteristics of probable covid-19 victims by rapid molecular testing

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Abstract. The capacity to establish the diagnosis among probable COVID 19 cases is limited, moreover, in death cases. Rapid molecular test (RMT) could be an alternative for establishing the definitive diagnosis in mortality cases, furthermore, management of bodies can be carried out in hospitals so as not to pollute the environment. The study was conducted in 6 hospitals and 10 primary health care centers in Mataram, West Nusa Tenggara from March 25th to August 5th, 2020. Nasopharyngeal swab post-mortem was done between 1-12 hours post-somatic dead in patients who were diagnosed with probable COVID-19. RMT was performed by GeneXpert Cepheid with cartridge Xpert Xpress SARS-CoV-2. The identification procedure was done by following the guideline in the manual procedure of the cartridge. The result of RMT will be positive or negative depending on the View Results Window of the GeneXpert System. There were 33 post-mortems nasopharyngeal swab samples that fulfill the criteria in the study period. Thirteen (39,39%) were positive for SARS-Cov-2. Most of the positive sample was taken 1-4 hours after somatic death, followed by 8-12 hours after somatic death consecutively 11 (84,61%) and 2 (15,38%). Among the positive cases, most of them were female, the age more than 60 years old, and owing to hypertension and cardiovascular disease as a comorbid sequentially 8 (61,53%), 8 (61,53%), and 5 (38,46%). RMT could be used as an alternative to identify the SARS-CoV-2 genetic material in the somatic dead patient until 12 hours post-mortem, however, the best time for taking a swab sample should be 1-4 hours post-mortem.

Keywords: Rapid Molecular Test, post mortem swab, Covid-19

1. Introduction

The Data from the Indonesian COVID-19 National Task Force, up until September 2020 shows the total number of COVID-19 cases in Indonesia is 172.053, with 7.343 (4.26%) death cases [1]. Moreover, in West Nusa Tenggara, as of September 11th 2.922 cases of confirmed COVID-19 have been reported, with 172 (5.89%) death cases [2].

The mortality data for probable COVID-19 cases is higher than the confirmed COVID-19 case. Some of the possible reasons are the progression of the disease that is getting worse, being delay in treatment, interventions, and early detection through tracing cases that have not been maximal [3]. The impact of



increasing deaths on probable COVID-19 cases from a social perspective is confusion in the funeral process of the corpse which ends with the forced collection of the body by family members [4,5].

Currently, there are several methods to detect the SARS-CoV-2 virus, by examining the antigen and its antibodies. Antigen method can be carried out by real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) and rapid molecular test (RMT) methods, while examination of sarCov2 virus antibodies is conducted by serological tests. Serology and RMT tests require less than 1 hour of examination, while RT-PCR takes longer [6]. The sensitivity of RT-PCR examination in the initial infection of SARS-CoV-2 is 83.3% [7].

The authors' found that the main influencing factor of people opting for RMT over RT-PCR testing is the time taken to obtain the results. This observation suggests the use of RMT for a post mortem rapid diagnostic alternative. If the body is proven to be COVID-19, handling the body will be carried out in a hospital which has a wastewater disposal facility so that the environment is not polluted.

2. Methodology

This study is an observational study with a cross-sectional design. The study was conducted from March 25 to August 5, 2020, in 6 hospitals and 10 health centers in Mataram City, West Nusa Tenggara. The sample taken was the corpse of probable COVID-19 cases, based on the clinical features and history taking. Informed consent was obtained from the families of the deceased patients. The number of samples was 33 bodies from a total of 58 probable COVID-19 bodies.

Postmortem swab sampling is carried out between 1 hour and 12 hours from somatic death by trained staffs with the following technical steps: extending the head in the neck joint, inserting a swabbing device into the nasopharynx for 20-30 seconds, inserting the tip of the swab into the virus transfer medium, sending it to the laboratory. The RMT used is the RMT machine for the detection of tuberculosis and haemophilus influenza. This machine can be used to detect the SARS-COV-2 virus using the GeneExpert engine. The automation process of sample preparation, RNA extraction, and result reading is integrated into the machine. This RMT uses a GeneXpert Cepheid machine with an Xpert Xpress SARS-CoV-2 cartridge, guided by following the procedure written in the instrument user manual. The examination results are displayed in the GeneXpert System window. The results will be obtained after about an hour

3. Results and discussion

During the study period, 58 probable covid-19 were found, however, only 33 (56.89%) patients were willing to sign the postmortem swab consent and RMT examination. Complete characteristics of the 33 patients who were subjected to post mortem swab and RMT examination can be seen in Table 1.

Tabel 1. Characteristics of the post-mortem swab by sex and age group.

Characteristics	RMT Results		Total N (%)
	Positive N (%)	Negative N (%)	
Sex			
Male	5 (15,2)	15 (45,4)	20 (60,6)
Female	8 (24,2)	5 (15,2)	13 (39,4)
Age(years old)			
> 60	8 (24,2)	9 (27,3)	17 (51,5)
51-60	3 (9,1)	6 (18,2)	9 (27,3)
41-50	1 (3,0)	3 (9,1)	4 (12,1)
31-40	0 (0)	1 (3,0)	1 (3,0)
21-30	0 (0)	1 (3,0)	1 (3,0)
11-20	1 (3,0)	0 (0)	1 (3,0)
< 11	0 (0)	0 (0)	0 (0)

We found most of the probable COVID-19 death cases also carry secondary comorbidity such as hypertension and chronic heart failure, namely in 7 (21.2%) cases. However, there are also 20 (60.6%) cases who are without a known history of any comorbid diseases. The full results can be seen in Figure 1.

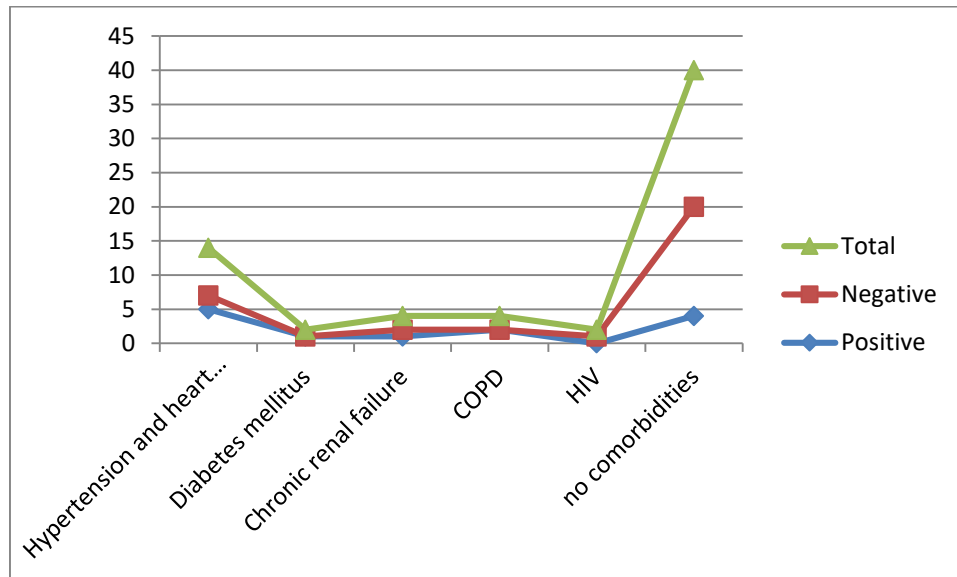


Figure 1. Characteristics of comorbidities among probable Covid-19 corpses.

RMT analysis found 13 cases with positive results of COVID-19 (39.4%). Most of the positive cases were found in the bodies that were analyzed 1-4 hours after death, there were no positive covid-19 cases in the 4-8 hour group, whereas in the 8-12 hours group there were 2 positive cases (6.1%). Complete data can be seen in Figure 2.

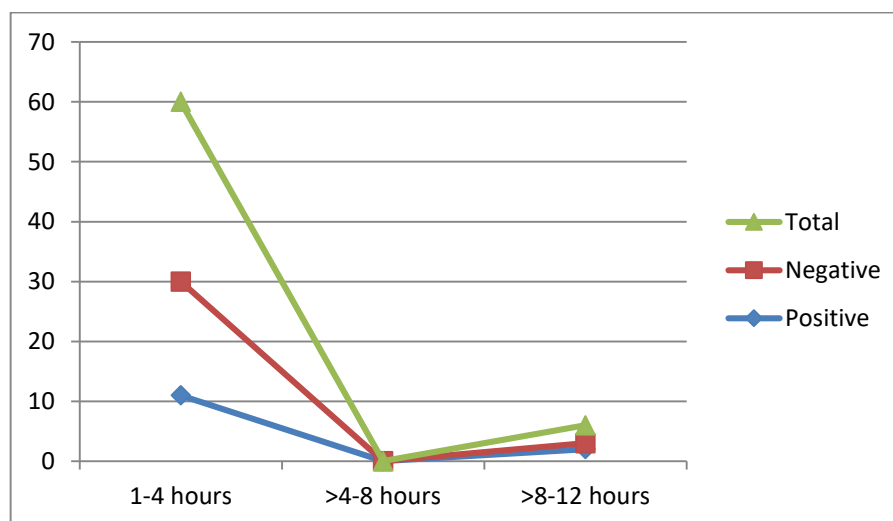


Figure 2. The result of RMT of probable Covid-19 corpses.

Early detection is very important to determine patient handling steps and disease outbreak control. The early clinical phase of COVID-19 does not show specific signs and symptoms. Furthermore, it shows similar symptoms as other respiratory infections [8].

RT-PCR is the recommended method for diagnosing the COVID-19 infection. However, the weakness of this test is that it requires staff expertise and adequate laboratory equipment. The problems that are often faced are the lack of supply of reagents, lack of laboratory instruments, the inability of staff to carry out complex laboratory techniques, shortage of skilled staff, expensive and time-consuming. Based on these reasons, a rapid test with high validity and accuracy is needed in detecting the SARS-CoV-2 virus antigen [9, 10, 11]. Based on local customs and religions on the island of Lombok that carry out funerals less than 24 hours after death, RMT was chosen to accelerate the status of the body, covid or not. The corpse of COVID-19 will be handled in the hospital, while non-COVID-19 will be handled at home. The family will not forcibly take the body from the hospital. These provisions are described in the standard operating procedures in the mortuary.

One example of a COVID-19 rapid test is the COVID-19 Ag Respi-Strip (CORIS Bioconcept, Gembloux, Belgium). This rapid antigen testing has a sensitivity of 30.2%. In general, this test cannot be used as a single diagnostic method in diagnosing COVID-19 because the viral load factor determines the test results [10]. With the same tool, Sidonie Lambert-Niclot et al obtained a sensitivity of 50%. The recommendation suggested that this kit is to be used several days after the onset of infection so that the viral load in the nasopharynx reaches a peak [8]. In general, the rapid molecular testing of choice is the test that produces results quickly in less than 1-2 hours, can diagnose COVID-19 in the early phase, technically simple, easy to read, and available in health facilities [9].

Although the results of molecular testing have high accuracy, they also have false-negative or false-positive results [9]. False positives can occur due to the cross-contamination of samples during the sample collection, pipetting, and processing processes or due to technical errors in the inspection. While false negatives can occur due to errors in sample collection, storage, transfer, purification, and processing. Apart from that, the yield depends on the quality of RNA extracted from the swab, genome mutation, and RNA degradation. Viral load and diagnostic timing also determine the results of molecular testing. Viral density and biodistribution of the SARS-CoV-2 virus were mostly found in bronchoalveolar lavage fluid, sputum, nasal swabs, and pharyngeal. Furthermore, as the disease progresses, the easier way to detect the virus in which peak density is found on the fourth day. In the early stages of infection where there are still few symptoms of the disease, a nasal or nasopharyngeal swab can be taken for early detection of the patient because the virus can be detected one week before symptoms develop. Factors that influence the differences in viral load in patients are an immune response, treatment effects, and epidemiological history [12].

The genetic diversity and evolution of the SARS-CoV-2 virus present its challenges in molecular diagnostics. The fourteenth percent of the SARS-CoV-2 variant could not be detected with at least one of the currently available primers. This results in a false negative result in molecular examination [12]. The sensitivity of the oligonucleotide primers and probes also determines test results such as unstable reagents, inappropriate amounts, irrational oligonucleotide design, and contaminants/impurities [12].

Thus, a negative result of molecular examination using either RT PCR or RMT does not mean that a person is not infected with the disease. Especially for RMT, some errors that need to be considered are errors in sampling, transportation, and handling. Meanwhile, errors in sample processing are likely low because all processes are run in machines and single-use disposable cartridges [13].

The RMT used in Indonesia is the RMT machine used for the detection of tuberculosis and Haemophilus influenza [14]. This machine can be used to detect the SARS-COV-2 virus using the GeneExpert engine and a special cartridge for the detection of the virus. The automation process of sample preparation, RNA extraction, and result reading is integrated into the machine. The validity and accuracy of the RMT are the same as for the RT-PCR [13].

Based on the results of RMT in this study, the largest positive percentage was obtained in sampling between 1-4 hours in 11 (84.61%) samples and 8-12 hours of sampling in 2 samples (15.38%). The human coronavirus can remain infectious on surfaces for up to 9 days [15]. The COVID-19 virus has been detected for up to 72 hours under experimental conditions [16,17]. A positive result on molecular examinations shows that the viral genetic material is detected by PCR or RMT, without being able to determine whether the virus is alive or dead. Viral culture is the only way to make sure the virus is alive

[18,19]. Since culture facilities remain inadequate, the authors argue that the principle of vigilance in handling probable COVID-19 bodies takes precedence over having to suppose the virus is alive or dead.

Despite limited scientific findings in regards to the deceased body of COVID-19 patients who are still infectious, the guidelines for handling COVID-19 bodies around the world apply the precautionary principle of transmitting the disease [20]. The principle of preparing and packing the body for transfer is to prevent bodily fluids from escaping from the body bag, clean contamination of body fluids, reducing the act of turning the body over and over [16]. The SARS-CoV-2 virus is classified in the Hazard Group 3, therefore, the risk of contamination in the mortuary is very serious so it should encourage us to be more careful when handling potentially infected people or materials [21]. In this study, 33 bodies were examined using the RMT method, where we found positive results on 13 (39.39%) bodies. Of the 13 bodies, 8 (61.53%) were women and 8 (61.53%) bodies were over 60 years old and 5 (38.46%) had hypertension and cardiovascular disease comorbidities.

The virus can survive on surfaces for hours or even days outside the body. Handling the corpse at home is highly discouraged because it pollutes the water and the environment. Therefore, it is recommended that the handling of the corpse be carried out in the hospital because it has standard wastewater disposal and available HEPA filter facilities [16,21].

The limitation of this study is the small number of samples, with 33 (56.89%) from 58 bodies. This is because it is difficult to get postmortem swab consent from the family members. Religious and cultural considerations were known to be the main factors for this rejection. Moreover, they are not willing if the body is treated according to the new health protocol

Conclusion

There were 13 (33.3%) probable COVID-19 bodies that were confirmed to have COVID-19 based on the RMT testing method. RMT can be an alternative for detecting the genetic material of the SARS-CoV-2 virus up to 12 hours after somatic death. Handling of bodies in hospitals reduces water and environmental pollution. One of the limitations of this study is the small number of samples, therefore further research is needed, especially concerning the characteristics of corpses suffering from COVID-19.

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