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Diagnostic Value of Saliva RT-PCR Test within Suspected SARS-CoV-2 Cases in Indonesia

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Abstract

Introduction: The ongoing SARS-CoV-2 pandemic has profoundly emphasized the pressing need for accurate and reliable diagnostic procedures. Given the potential health risks associated with nasopharyngeal swabs, there has been growing interest in seeking alternative diagnostic mediums. In this context, our study delved into evaluating saliva as a potential diagnostic tool, simultaneously assessing its efficiency in relation to patient demographics and their exhibited clinical symptoms. **Methods:** Spanning from May to December 2020, we conducted a comprehensive cross-sectional analysis. We meticulously examined medical records to gather insights on patient characteristics, existing health conditions, onset of symptoms, clinical manifestations, and compared the results obtained from both salivary and nasopharyngeal RT-PCR tests for SARS-CoV-2. **Results:** Among the individuals suspected of SARS-CoV-2 infection, the mean age stood at 52.4 years, with males representing 60.3% of this group. Interestingly, a significant 76.9% reported underlying health conditions, predominantly hypertension and diabetes. The most commonly reported symptoms encompassed respiratory challenges, notably coughing and shortness of breath, succeeded by symptoms like nausea, fever, and a general sense of fatigue. The performance of saliva tests, in terms of accuracy, appeared to be significantly influenced by the timing of symptom emergence. **Conclusion:** The RT-PCR tests utilizing saliva samples demonstrated considerable promise, especially during the early stages of symptom manifestation, providing a reliable alternative to traditional nasopharyngeal swabs. The findings suggest a superior diagnostic sensitivity when utilizing saliva during the initial phases of a SARS-CoV-2 infection.

Keywords: SARS CoV-2, RT-PCR, Saliva, Nasopharyngeal swab.

Abstrak

Pendahuluan*: Pandemi SARS-CoV-2 yang berlangsung telah menekankan secara mendalam kebutuhan akan prosedur diagnostik yang akurat dan dapat diandalkan. Mengingat risiko kesehatan yang mungkin berhubungan dengan usap nasofaring, minat untuk mencari medium diagnostik alternatif semakin meningkat. Dalam konteks ini, tujuan penelitian kami mengevaluasi air liur sebagai alat diagnostik potensial, seraya menilai efisiensinya sehubungan dengan demografi pasien dan gejala klinis yang mereka tunjukkan.* ***Metode:*** *Meliputi periode dari Mei hingga Desember 2020, kami melakukan analisis lintas-seksi yang komprehensif. Kami secara teliti memeriksa catatan medis untuk mendapatkan wawasan mengenai karakteristik pasien, kondisi kesehatan yang ada, awal munculnya gejala, manifestasi klinis, dan membandingkan hasil yang*

diperoleh dari tes RT-PCR saliva dan nasofaring untuk SARS-CoV-2. ***Hasil:*** *Dari individu yang dicurigai terinfeksi SARS-CoV-2, usia rata-rata adalah 52,4 tahun, dengan pria menyumbang 60,3% dari grup ini. Menariknya, sebanyak 76,9% melaporkan kondisi kesehatan yang mendasari, terutama hipertensi dan diabetes. Gejala yang paling sering dilaporkan meliputi masalah pernapasan, terutama batuk dan sesak napas, diikuti oleh gejala seperti mual, demam, dan rasa lelah secara umum. Kinerja tes air liur, dalam hal akurasi, tampaknya dipengaruhi secara signifikan oleh waktu kemunculan gejala.* ***Kesimpulan:*** *Tes RT-PCR yang menggunakan sampel air liur menunjukkan prospek yang cukup menjanjikan, terutama selama tahap awal manifestasi gejala, menyediakan alternatif yang dapat diandalkan untuk usap nasofaring tradisional. Temuan menunjukkan sensitivitas diagnostik yang lebih unggul ketika menggunakan air liur pada fase awal infeksi SARS-CoV-2.*

Kata Kunci: *SARS CoV-2, RT-PCR, Saliva, Usap Nasofaring.*

I. INTRODUCTION

Coronavirus disease 2019 (SARS COV-2) due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2) has continued to increase since the World Health Organization (WHO) announced an outbreak in Hubei province and the Chinese city of Wuhan in December 2019.[1] Since then, the number of SARS COV-2 cases in Indonesia has continued to increase. The latest data in Indonesia on March 4, 2022, based on the Ministry of Health of the Republic of Indonesia, recorded 5,667,355 positive cases and 531,696 active cases.[2]

Early detection is useful to control the progression of SARS COV-2. Since the beginning of the pandemic, WHO has recommended a diagnostic method by detecting viral RNA using real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on previous experience method for detecting respiratory tract infections.[3] The first choice of RT-PCR examination recommended by WHO is to use a nasopharyngeal swab sample specimen. However, RT-PCR examination of the nasopharynx takes several hours to a day to achieve results, making it difficult to conduct a rapid and mass examination. Nasopharyngeal swab sampling procedure involves close contact between health workers. It can stimulate irritation of the pharynx, cough and runny nose which can lead to the formation of transmission through aerosols thus increase risk transmission.[4]

Several studies have found another method of diagnosing SARS COV-2, namely by using specimens of other body fluids such as tears, saliva, urine and feces.[5] Salivary examination has been the diagnosis of choice since the SARS outbreak in 2003 and has been considered for the diagnosis of Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV).[6] Salivary diagnostic is an alternative option that is easy to use, portable, provide results within 30 to 60 min and can be performed by non-specialized medical staff.[4] In contrast to nasopharyngeal examination, the results of saliva examination are believed to depend on the time of sampling and onset patients.[7]

Currently there is still not enough research to assess salivary examination compared to nasopharyngeal examination in Indonesia, therefore the researcher aims to compare salivary examination as a diagnostic tool with characteristics, onset, and clinical symptoms in patients confirmed positive for SARS COV-2.

II. MATERIAL AND METHODS

A cross-sectional study was conducted at Persahabatan National Respiratory Center Hospital (NRRH) from May to December 2020. This research have Institutional Review Boards (IRBs) of Persahabatan Hospital Approval no 81/KEPK-RSUPP/8/2020. Data were obtained from patient medical records. The data collected in the form of subject characteristics, comorbidities, onset, symptoms, laboratory examinations and results of salivary and nasopharyngeal examinations RT-PCR CoV-2.

Sample collection was carried out using the consecutive sampling method, namely patients with suspected SARS COV-2 aged more than 18 years who were treated at the Persahabatan NRRH during the study period and were willing to sign the consent form to participate in the study. All data were collected, stored and processed using Excel and SPSS version 25. Scaled variable with normal and abnormal distribution were expressed as mean and median values, then were tested using the unpaired independent t-test and the Mann-Whitney test. Categorical data were analyzed using the Pearson Chi-square test or Fisher's exact test.

III. RESULTS

Based on the inclusion and exclusion criteria, the number of study subjects was 78 patients consisting of positive salivary patients (n=34) and negative salivary patients (n=44) as shown in table 1. Positive salivary patients consisted of 23 men (67.6%) and 11 women (32.4%). In patients with negative salivary results, there were 24 males (54.5%) and 20 females (45.5%). The age range of positive salivary subjects was between the ages of 23 to 78 years with a mean age value of 52.4 years, while the age range of negative salivary subjects was between 24 to 74 years with an average age value of 53 years. No correlation was found between the age and sex characteristics of the subjects with saliva examination. Hypertension was the most common comorbid in all cases (46.2%). Hypertension tend to appear in saliva negative subjects (56.8%). Diabetes mellitus was the second comorbid with a total of 30 cases (38.5%).

TABLE 1. CLINICAL CHARACTERISTICS AND LABORATORY FINDINGS OF SUSPECTED SARS COV-2 CASES.

Characteristics	Total		Confirmed Saliva Positive		Confirmed Saliva Negative		p
	n= 78	%	n= 34	%	n= 44	%	
Age (SD)		52.4 (3.03)		51.5 (4.13)		53 (4.09)	0.68
Age (range)		23-78		23-78		24-74	
Sex							
Male	47	60.3%	23	67.6%	24	54.5%	0.34
Female	31	39.7%	11	32.4%	20	45.5%	
Comorbidities	60	76.9%	26	76.5%	34	77.3%	1.00
Hypertension	36	46.2%	11	32.4%	25	56.8%	0.55
Diabetes Mellitus	30	38.5%	15	44.1%	15	34.1%	0.50
Respiratory Symptoms	61	78.2%	26	76.5%	35	79.5%	0.96
Cough	56	71.8%	23	67.6%	33	75.0%	0.64
Runny nose	8	10.3%	3	8.8%	5	11.4%	1.00
Dyspnea	51	65.4%	23	67.6%	28	63.6%	0.90
Gastrointestine Symptoms	46	59.0%	21	61.8%	25	56.8%	0.84
Sore throat	15	19.2%	6	17.6%	9	20.5%	0.98
Nausea	41	52.6%	21	61.8%	20	45.5%	0.23
Diarrhea	8	10.3%	2	5.9%	6	13.6%	0.45
Non-Gastrointestine Symptoms							
Fever	41	52.6%	18	52.9%	23	52.3%	1.00
Headache	13	16.7%	7	20.6%	6	13.6%	0.61
Shiver	4	5.1%	1	2.9%	3	6.8%	0.63
Fatigue	33	42.3%	14	41.2%	19	43.2%	1.00
Muscle ache	2	2.6%	1	2.9%	1	2.3%	1.00
Stomach ache	6	7.7%	2	5.9%	4	9.1%	0.69
WHO Severity Scale							
Mild/Severe							
Severe (>3)	33	42.3%	16	47.1%	17	38.6%	0.61
Mild (0-3)	45	57.7%	18	52.9%	27	61.4%	
Oxygen Therapy							
Oxygen Therapy Needed (>4)	4	5.1%	3	8.8%	1	2.3%	0.31
Without Oxygen Therapy (0-4)	74	94.9%	31	91.2%	43	97.7%	
High Oxygen Ventilation							
High Flow Oxygen Needed (>5)	1	1.3%	0	0.0%	1	2.3%	1.00
Without High Oxygen (0-5)	77	98.7%	34	100.0%	43	97.7%	
Laboratory Findings	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	
Hemoglobin (g/dL)	75	12.9 (95%CI: 12.4-13.4)	33	13.7 (95%CI: 13.1-14.2)	42	12.3 (95%CI: 11.5-13.1)	-
Hemoglogin <13.7g/dL (male) or <11.9g/dL (female)	34	45.3%	11	33.3%	23	54.8%	0.18
Hematocrit (%)	76	38 (95%CI: 36.6-39.5)	33	39.6 (95%CI: 37.9-41.4)	43	36.8 (95%CI: 34.6-39.1)	0.07
Eritrosit (10^6/µl)	76	5.7 (95%CI: 3.3-8.0)	33	7.4 (95%CI: 2.0-12.9)	43	4.3 (95%CI: 4.0-4.6)	0.20
Leukosit (10^3/µl)	76	9.9 (95%CI: 8.7-11.1)	33	9.3 (95%CI: 7.3-11.3)	43	10.4 (95%CI: 8.9-11.9)	0.39
<4.000		3/76 (3.9%)		1/33 (3.0%)		2/43 (4.7%)	-
4.000-10.000		49/76 (64.5%)		25/33 (75.8%)		24/43 (55.8%)	-
>10.000		24/76 (31.6%)		7/33 (21.2%)		17/43 (39.5%)	-
Trombosit (10^3/µl)	76	291.7 (95%CI: 267.6-315.7)	33	271.5 (95%CI: 244.0-298.9)	43	307.2 (95%CI: 270.7-343.6)	0.15
Basofil (%)	76	0.29	33	0.30	43	0.28	0.83

		(95%CI: 0.20-0.37)		(95%CI: 0.18-0.41)		(95%CI: 0.14-0.41)	
Eosinofil (%)	76	1.47	33	1.29	43	1.61	0.42
		(95%CI: 1.09-1.86)		(95%CI: 0.75-1.84)		(95%CI: 1.07-2.15)	
Neutrofil (%)	75	70.85	32	73.26	43	69.06	0.27
		(95%CI: 67.15-74.56)		(95%CI: 69.13-77.40)		(95%CI: 63.40-74.72)	
<40%		2/75 (2.7%)		0/32 (0.0%)		2/43 (4.7%)	-
40-60%		9/75 (12.0%)		3/32 (9.4%)		6/43 (14.0%)	-
>60%		64/75 (85.3%)		29/32 (90.6%)		35/43 (81.4%)	-
Limfosit (%)	76	18.75	33	16.72	43	20.31	0.28
		(95%CI: 15.54-21.96)		(95%CI:13.56-19.89)		(95%CI: 15.20-25.42)	
<20%		51/76 (67.1%)		23/33 (69.7%)		28/43 (65.1%)	-
20-40%		22/76 (29.0%)		10/33 (30.3%)		12/43 (27.9%)	-
>40%		3/76 (3.9%)		0/33 (0.0%)		3/43 (7.0%)	-
Monosit (%)	76	8.13	33	8.03	43	8.2	0.84
		(95%CI: 7.31-8.95)		(95%CI: 6.76-9.31)		(95%CI: 7.11-9.29)	
LED (mm/hour)	32	59.1	16	55.9	16	62.4	0.61
		(95%CI: 47.1-71.2)		(95%CI: 39.0-72.8)		(95%CI: 44.7-80.0)	
Bilirubin (mg/dL)	58	0.9	28	0.9	30	0.9	0.97
		(95%CI: 0.7-1.2)		(95%CI : 0.6-1.3)		(95%CI: 0.6-1.3)	
Ureum (mg/dL)	62	35.8	28	30.6	34	40	0.13
		(95%CI: 29.7-41.9)		(95%CI: 22.3-38.9)		(95%CI: 31.4-48.6)	
Creatinin (mg/dL)	63	1.2	28	0.9	35	1.4	0.10
		(95%CI: 0.9-1.4)		(95%CI: 0.8-1.1)		(95%CI: 0.9-1.8)	
Post Symptom Onset of Saliva sample (day)	**n=78**	**%**	**n=34**	**%**	**n=44**	**%**	
3 days sample							
0-3 days	23	29.5%	13	38.2%	10	22.7%	0.22
>3 days	55	70.5%	21	61.8%	34	77.3%	
4 days sample							
0-4 days	27	34.6%	17	50.0%	10	22.7%	0.02
>4 days	51	65.4%	17	50.0%	34	77.3%	
5 days sample							
0-5 days	35	44.9%	19	55.9%	16	36.4%	0.14
>5 days	43	55.1%	15	44.1%	28	63.6%	
6 days sample							
0-6 days	41	52.6%	21	61.8%	20	45.5%	0.23
>6 days	37	47.4%	13	38.2%	24	54.5%	

Clinical symptoms divided into three main group, namely respiratory symptoms, gastrointestinal symptoms and non-gastrointestinal symptoms. Respiratory symptom found in most cases (78.2%). Cough was a respiratory symptom reported by subjects (71.8%), but no significant difference was found between positive salivary subjects and negative saliva swab results. Dyspnea was the second symptom that is often complained of, about 51 cases (65.4%) of the total subject population. Runny nose was a respiratory symptom that the subject complained a little about, which was only 8 patients (10.3%). Gastrointestinal symptoms with the most complaints were nausea (52.6%) followed by sore throat (19.2%) and diarrhea (10.3%). Non-gastrointestinal symptoms that were often complained of are fever (52.6%), fatigue (42.3%), headache (16.7%), stomach-ache (7.7%), shiver (5.1%) and muscle-ache (2.6%). WHO severity assessment show that 57.7% cases within mild scale with 94.9% without oxygen therapy and 1.3% need high flow oxygen.

Laboratory findings showed that anemia was observed 45.3% of all cases. Most anemia cases was found on negative saliva results (54.8%). Lymphopenia occurred on 67.1% cases, 85.3% High neutrophil count and 64.3% patient has white blood cell (WBC) count around 4.000-10.000 in all cases.

Salivary examination based on post symptom onset in all suspected case was shown in Table 1. Correlation was found on the 4th day post symptom onset (p= 0.02). There were 50% positive and 22.7% negative cases found in 0-4 days post symptom onset. Salivary examination on days >4 post symptom onset results in 50% and 77.3% in all saliva positive and negative respectively. There was no correlation found on the 3rd day (p=0.22), 5th day (p=0.14) and 6th day

(p=0.23). Meanwhile saliva RTPCR results of confirmed SARS-CoV2 cases was significant in 4th (p=0.01) and 5th (p=0.04) post symptom onset shown in Table 2.

TABLE 2. POST SYMPTOM ONSET OF CONFIRMED SARS COV-2 CASES.

Characteristics	Total		Confirmed Saliva Positive		Confirmed Saliva Negative		p
	n= 72	%	n= 33	%	n= 39	%	
Saliva Sample based on onset time (day)							
3 days sample							
0-3 days	21	29.2%	13	39.4%	8	20.5%	0.14
>3 days	51	70.8%	20	60.6%	31	79.5%	
4 days sample							
0-4 days	25	34.7%	17	51.5%	8	20.5%	0.01
>4 days	47	65.3%	16	48.5%	31	79.5%	
5 days sample							
0-5 days	31	43.1%	19	57.6%	12	30.8%	0.04
>5 days	41	56.9%	14	42.4%	27	69.2%	
6 days sample							
0-6 days	37	51.4%	21	63.6%	16	41.0%	0.94
>6 days	35	48.6%	12	36.4%	23	59.0%	

IV. DISCUSSION

We analysed demographical characteristics, comorbidities, presenting symptoms, laboratory findings and correlation saliva sampling based on time onset. In this study, was to find the association of salivary examination with patient demographic characteristics. In all confirmed SARS COV-2 patients, the mean age was 52.4 years. Male gender was found more often (60.3%) than female gender (39.7%). This is in line with previous studies where confirmed male patients were more often found.[2,8,9]

Comorbidity is a risk factor that causes increased mortality in SARS COV-2 cases. Within all suspected cases, Hypertension and type II diabetes are the most common. This was similar to the finding prom previous study.[1,10] There are various symptoms that appear, most of the respiratory symptoms, namely cough and dyspnoea was present. Other symptoms of were fever, nausea and fatigue. This finding was in line with previous studies that discovered similar symptoms of SARS CoV-2.[8,11-14] These symptoms are often an indication for patients to be hospitalized.[15]

Patients with lymphopenia was common in all suspected cases (67.1%). The result was similar in previously studies. It was suggested that SARS CoV-2 might act on lymphocytes as does SARS-CoV. Particle of the virus would infect through respiratory mucosa then could infect other cells, induce cytokine storm, generate series of immune responses and cause change in immune cells.[13]

Several studies found the similar accuracy of salivary RT-PCR examination compared to nasopharyngeal/oropharyngeal examination.[16] In some cases, the number of salivary examination results can be equal to or higher than the nasopharyngeal/ oropharyngeal examination. When comparing saliva with the gold standard nasopharyngeal swab, various sensitivity values were found with an average sensitivity value of 85% while most rate the specificity level to be >90%.[17] Previous study found that saliva has higher positivity rate by compare self-collected saliva sample and health care worker nasopharyngeal swab, the percent of positive rate was 93.8% (95%CI, 86.0-97.9%) dan 86.3% (95%CI, 76.7-92.8%) respectively.[18] Other study found that salivary examination had a lower diagnostic value than the nasopharynx/oropharynx because false-positive values are often found in patients with a typical clinical or radiological appearance and negative nasopharyngeal swab.[4] However, nasopharyngeal swab examination is often associated with false negative values up to 30% after the onset of symptoms so that it can be a misclassification to assess the accuracy of saliva analysis studies.[7]

The study found that saliva examination was related to post symptom onset, namely on 4th (p=0.02) in suspected cases, while day 4th day (p=0.01) and 5th day (p=0.04) in

confirmed cases. In line with the results of the study, previous study found the timing of saliva collection based on onset is believed to affect the results of the examination.[19,20] Previous study found salivary examination sensitive on first week onset.[19] Others found on days 1-5 of onset of saliva test has an average sensitivity value of 81%, which is greater than nasopharynx sensitivity value of 71%.[20]

V. CONCLUSION

The study found that saliva RT-PCR examination provided relevant results at the beginning of symptom onset and was reliable for detecting negative nasopharyngeal swab results, thus increasing the sensitivity to diagnose SARS CoV-2.

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CONFLICT OF INTEREST

None

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