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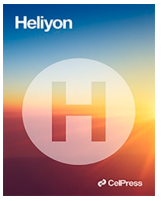

Research article

Dry eye symptoms are prevalent in moderate-severe COVID-19, while SARS-COV-2 presence is higher in mild COVID-19: Possible ocular transmission risk of COVID-19

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ABSTRACT

Purpose: To evaluate the correlation between dry eye symptoms and coronavirus disease 2019 (COVID-19) infection and to assess the real-time reverse transcription–polymerase chain reaction (RT−PCR) of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) from the conjunctival swab.

Methods: A prospective observational case series study was conducted of all suspected and confirmed COVID-19 patients from Dr. Cipto Mangunkusumo Hospital (RSCM) and the Universitas Indonesia Hospital (RSUI). On the first day of the visit (day 0), systemic clinical symptoms and naso-oropharyngeal (NO) RT−PCR results will classify all subjects as non-, suspected, or confirmed (mild, moderate, and severe) COVID-19. In all patients, we determined the dry eye symptoms based on the Ocular Surface Disease Index (OSDI) and followed up 7(day 7) and 14 days (day 14) after the first visit. When it was technically possible, we also examined the objective dry eye measurements: tear meniscus height (TMH), noninvasive Keratograph® break-up time (NIKBUT), and ocular redness. Additionally, we took conjunctival swab samples for SARS-CoV-2 RT-PCR in all patients.

Results: The OSDI scores for 157 patients decreased across days 0, 7, and 14 (median (interquartile range): 2.3 (0–8), 0 (0–3), and 0 (0-0), p value < 0.0001 (D0 vs D14). The moderate-severe COVID-19 group had a higher OSDI score than the other groups at median D0 (15.6 vs 0–2.3), p value < 0.0001 and this pattern was consistently seen at follow-up D7 and D14. However, dry eye complaints were not correlated with the three objective dry eye measurements in mild-moderate COVID-19 patients. NO RT−PCR results were positive in 32 (20.4%) patients, namely, 13 and 19 moderate-severe and mild COVID-19 patients, respectively. Positive RT−PCR

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results were observed in 7/157 (4.5%) conjunctival swab samples from 1 in non-COVID-19 group and 6 in mild group.
Conclusion: In the early phase of infection, COVID-19 patients experience dry eye symptoms, which have no correlation with objective dry eye measurements. SARS-CoV-2 in conjunctival swab samples can be detected in patients with normal-to-mild COVID-19, which shows the risk of ocular transmission.

Abbrevations

SARS-CoV-2	Severe Acute Respiratory Syndrome-Corona Virus-2
COVID-19	Corona-virus Disease 2019
OSDI	Ocular Surface Disease Index
TMH	tear meniscus height
NIKBUT	non-invasive Keratograph® break-up time
RT-PCR	Reverse Transcription – Polymerase Chain Reaction
NO	nasopharyngeal/oropharyngeal
ARDS	acute respiratory distress syndrome
ACE2	angiotensin-converting enzyme 2
TMPRSS2	transmembrane serine protease 2
ANPEP	alanyl aminopeptidase
DPP4	dipeptidyl peptidase 4
NRP1	neuropilin 1

1. Introduction

In December 2019, coronavirus disease 2019 (COVID-19), a highly contagious infection caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), emerged in Wuhan, China, and this novel virus swiftly traversed the globe [1]. The clinical spectrum of COVID-19 is wide-ranging, encompassing individuals who are asymptomatic, those with mild self-limiting respiratory tract symptoms, others exhibiting severe progressive pneumonia, and in the most critical cases, patients who develop acute respiratory distress syndrome (ARDS) [2]. Additionally, a small proportion of patients have extrapulmonary and atypical clinical manifestations, which can lead to a delayed diagnosis. Consequently, these asymptomatic or atypical extrapulmonary symptomatic individuals contribute to person-to-person transmission due to the absence of typical symptoms or awareness [3–5].

Related to person-to-person transmission, one route of viral infection that has gained attention involves the eye. The ocular surface represents one of the extrapulmonary sites that has emerged as a potential reservoir for SARS-CoV-2 infection. Due to its anatomy, the ocular surface is exposed to the environment, rendering it susceptible to SARS-CoV-2 infection, which might lead to a systemic infection via a nasolacrimal duct or by touching the nose with hands contaminated with the virus from tears [6]. Supporting this hypothesis is an experimental study of nonhuman primates, demonstrating that SARS-CoV-2 inoculation of the ocular conjunctiva causes interstitial pneumonia mirroring mild COVID-19 [7]. Although the overall prevalence of ocular manifestations is low, several receptors (angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2), alanyl aminopeptidase (ANPEP), dipeptidyl peptidase 4 (DPP4), and neuropilin 1 (NRP1)) expressed on the ocular surface might allow SARS-CoV-2 to replicate and act as a gateway for transmitting the virus to extraocular sites to establish a respiratory infection [8,9].

The possibility of ocular surface involvement warrants the search for ocular manifestation. A study has examined one of the ocular surface subjective symptoms using the Ocular Surface Disease Index (OSDI) questionnaire, up to 40% of COVID-19 patients report ocular problems, including complaints of soreness and other dry eye symptoms [10]. Additionally, a literature review has shown decreased tear production in COVID-19 patients after the infection subsides [11]. This condition might be related to coronavirus' properties of neurotropism and neuroinvasiveness rather than ocular surface tropism; these properties might affect the sympathetic and parasympathetic nerve fibers to the lacrimal glands to cause a decrease in tears [12]. In addition, during SARS-CoV-2 infection, gut dysbiosis might occur, leading to an imbalance in the immune response through gut-immune system crosstalk. Alterations in the gut may lead to changes in the composition of the tear film, which can contribute to the development of dry eye disease by affecting the production of neurotransmitters and hormones involved in regulating tear secretion and ocular surface homeostasis [13–15].

Therefore, we tried to prove this concept by investigating the association between dry eye symptoms, as assessed through the Ocular Surface Disease Index (OSDI) questionnaire, and SARS-CoV-2 infection in individuals with varying degrees of COVID-19 disease severity. We aimed to understand the possible mechanisms of ocular surface involvement in COVID-19 and whether the ocular surface can serve as a potential viral transmission route to other body parts. Additionally, we sought to explore whether the prevalence of ocular symptoms, including dry eye-related complaints in COVID-19 patients, could be attributed to the dry eye pathogenesis mechanism. Furthermore, this research aimed to assess the diagnostic accuracy of detecting SARS-CoV-2 through conjunctival swab

samples. In summary, our study aimed to elucidate the relationship between COVID-19, ocular surface symptoms, and the potential role of the eye in the transmission and manifestation of the disease.

2. Material and methods

2.1. Study design and ethical clearance

We conducted a prospective observational study between July and December 2020. We evaluated dry eye symptoms using subjective measurements on their initial visit to the clinic for suspected COVID-19 namely day 0 (D0) and continued on days 7 (D7) and 14 (D14) post initial visit. Objective examinations of dry eye symptoms were carried out once on D0 along with conjunctival and nasopharyngeal/oropharyngeal (NO) swab sampling for reverse transcription–quantitative polymerase chain reaction (RT–qPCR).

This multicenter study was carried out at Dr. Cipto Mangunkusumo Hospital (RSCM) and Universitas Indonesia Hospital (RSUI) and was approved by a joint ethics committee of the Faculty of Medicine, University of Indonesia–Cipto Mangunkusumo Hospital, with the reference number KET-727/UN2.FI/ETIK/PPM.00.02/2020, and it was approved on July 13, 2020. The research followed the tenets of the Declaration of Helsinki, and written informed consent was obtained from all subjects.

2.2. Subjects

We included patients older than eighteen who either had symptoms suggestive of COVID-19 or confirmed COVID-19 who came to the respiratory clinics of RSCM and RSUI. All included patients were classified according to the fifth revision of the National Guidelines on COVID-19 Prevention and Control released by the Indonesian Health Ministry [16]. Patients with insufficient data, such as those who could not be contacted by phone or those who had passed away, were excluded.

Diagnosis of COVID-19 was confirmed with a positive real-time RT–PCR result for NO samples [17]. Based on the severity of the disease, we classified these patients as being asymptomatic or having mild, moderate, or severe disease. Asymptomatic individuals exhibited no symptoms. Patients with mild illness showed any signs or symptoms of COVID-19 but no shortness of breath, dyspnea, or COVID-19 typical chest X-ray (CXR) findings including bilateral peripheral and basal multifocal airspace opacities (ground-glass opacity (GGO) and consolidation [18]. Those with moderate illness were characterized by lower respiratory disease (fever, dyspnea, cough) during clinical assessment, GGO findings on CXR examination but no signs of severe illness. Finally, patients with severe illness exhibited lower respiratory disease and one of the following signs: respiratory frequency of >30 breaths/min or oxygen saturation (SpO2) $<90\%$ on room air at sea level [16].

Patients suspected of having COVID-19 had negative PCR results but presented with respiratory symptoms suggestive of COVID-19, such as flu, cough, fever within 14 days of exposure, and a travel history to highly endemic places with a high number of cases reported. We included those patients in the suspected group to anticipate false-negative results because of other transmission routes, such as fecal-oral and ocular transmission [19].

If the subject had no symptoms, a negative PCR from an NO swab, and no history of previous COVID-19, they were classified as non-COVID-19. These subjects underwent a PCR test for tracing after closely contacting a confirmed COVID-19 patient. Discharged COVID-19 patients were asymptomatic patients with negative NO PCR results who had been previously diagnosed with COVID-19, confirmed by a previous positive NO swab test performed within 1 month before the study. We grouped non-COVID-19 patients and discharged COVID-19 patients into the non-COVID-19 group due to clinical concordance of having no symptoms with a negative result based on the latest NO PCR test.

2.3. Ocular data collection

2.3.1. Subjective measurement

In this study, we used the OSDI questionnaire (Allergan Inc., Irvine, CA, USA) as the primary tool for evaluating dry eye symptoms. OSDI is a questionnaire consisting of 12 items, aiming to evaluate the symptoms related to ocular irritation that align with dry eye disease and ascertain their impact on vision-related capabilities [20]. Patients subjectively completed the OSDI questionnaire on D0, assisted by the doctors, and the investigators contacted them on D7 and D14 by telephone to follow up on their symptoms using the same questionnaire. The OSDI score derived from the patient-completed questionnaire were computed and categorized according to the subsequent OSDI score classification: normal (0–12.9), mild (13–22.9), moderate (23–32.9), and severe (33–100) [20].

2.3.2. Objective measurements

We examined objective dry eye signs using an advanced corneal topographer (Oculus Keratograph® 5 M Oculus GmbH, Wetzlar, Germany). We evaluated the tear meniscus height (TMH), the noninvasive Keratograph® break-up time (NIKBUT), and ocular redness on D0 [21].

2.4. SARS-CoV-2 RT–PCR of nasopharyngeal-oropharyngeal (NO) and conjunctival samples

2.4.1. Nasopharyngeal-oropharyngeal (NO) sample collection

RT–qPCR for NO samples is an obligatory procedure for patients with suspected COVID-19 before they receive therapy at our hospital. NO swab samples were collected based on an established method. Well-trained residents took the NO sample using a flocked

swab from both nasal cavities and the oropharynx. The swab is inserted in the nose horizontally, along an imaginary line between the nostril and the ear. Upon reaching the posterior wall of the nasopharynx, rotate the tip of the swab continuously for a few seconds, before gently removing the swab. Oropharyngeal sampling is easier to perform. The swab is directed toward the rear wall of the oropharynx and it is rotated a few times before removal. After taking the sample, insert both swabs in the same tube [22]. It was put into a cold viral transport medium, which was examined within 2 h after collection in the laboratories of both hospitals.

2.4.2. Conjunctival swab samples

The conjunctival swab technique was designed to collect tears and conjunctival secretions from patients. Well-trained doctors collected each conjunctival swab from both eyes. The subject was asked to look up, the lower eyelid was pulled down using a thumb, and the swab was rubbed over the inferior fornix from the medial to the lateral side [23]. The procedure is often painful, so we used Pantocain 0.5% eye drops before the swab and also to increase the chance of obtaining positive results [24]. After finishing the procedure, we dropped a few artificial tears to make the subject more comfortable. The swab was immersed in viral transport medium and stored at 4 °C before the sample was tested for SARS-CoV-2.

2.4.3. Sample transportation and RNA extraction

Samples were transported to the laboratory in a cool box containing ice gel within 4 h after swabbing. Samples were stored at −80 °C until they were used for PCR within 4 weeks. Samples were extracted using the Zymo Research RNA Viral Mini Kit (Irvine, CA, USA) according to the instructions with a final dilution of 50 μL. RNAs were used immediately for real-time PCR analysis.

2.4.4. Real-time PCR of SARS-CoV-2 in conjunctival swab samples

Real-time PCR was performed using CDC primers against the N1 and N2 SARS-CoV-2 genes. The RP gene was used as the internal control. We used the SensiFAST No ROX One-Step PCR Kit (Bioline, OH, USA) with a final volume reaction of 20 μL. As many as 4 μL of sample RNA was used as the PCR template. PCR was conducted on a Roche LightCycler 480. Thermal cycling followed the manual instructions from the PCR kit. All preparations were performed in a biosafety level II laboratory.

Positive samples showed increasing fluorescence signals for the N1, N, and RP genes. For ambiguous results (only N1 or N2 genes showed positive results), RdRP (Kogene Biotech, Seoul, Korea) was used for confirmation. RdRP dan N genes have conserved sequence regions that are suitable as PCR targets. RdRP gene is an RNA-dependent RNA polymerase gene located in the open reading frame ORF1ab region. The N gene is a nucleocapsid protein gene [22]. The confirmation assay was conducted according to the manual instructions. The ambiguous samples positive for RdRP were considered positive for SARS-CoV-2. PCR amplification results were analyzed using LightCycler 480 applications. Positive control, negative control, and nontemplate control (NTC) samples were always included per PCR run. A CT value > 40 was considered a negative result.

Two of the SARS-CoV-2-positive samples from conjunctival swabs underwent sequencing using the ARTIC network workflow on Oxford Nanopore's GridIon platform with >100x coverage. The samples were extracted and converted into complementary DNA (cDNA) and then PCR was performed to amplify overlapping "tiled" sections covering the whole SARS-CoV-2 genome. This amplification process utilized Integrated DNA Technologies (IDT) V3 primer pools, resulting in 400 bp amplicons with an approximate 20 bp overlap to cover the 30 kb SARS-CoV-2 genome in accordance with the ARTIC multiplex PCR protocol. NEBNext Ultra II End Repair/dA-Tailing Module (New England BioLabs) was used to perform the end repair of amplified cDNA. To enable sequencing, the amplified amplicons were tagged with unique barcodes using the Native Barcoding Expansion pack, facilitating the simultaneous preparation of 32 samples for sequencing in a single run. These barcoded samples were then combined or pooled for streamlined sequencing. Each individual sample designated for multiplex sequencing was affixed with a distinct native barcode. Subsequently, the next step involved the ligation of sequencing adapters to the pooled barcoded samples before the library was readied for Nanopore sequencing. The FASTQ files generated from GridIon platform were assembled and analyzed using ARTIC bioinformatic pipeline (https://github.com/artic-network/artic-ncov2019). The SARS-CoV-2 sequence data we obtained have been deposited to Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org/).

2.5. Statistical analysis

The OSDI distribution was calculated in Microsoft Excel for Mac (version 16.48). Statistical analysis was performed using SPSS Statistics for Mac, Version 23.0 (IBM SPSS, Armonk, NY, USA). A Friedman test was performed to analyze each group's OSDI score per week. Pearson's chi-square test was used to analyze the proportions of patients (mild, moderate, or severe) who had normal ocular conditions and dry eye disease symptoms based on the OSDI categories. Chi-square tests were performed and odds ratios were calculated to evaluate the correlation between positive NO PCR results and dry eye symptoms, conjunctival PCR results and dry eye symptoms, and NO and conjunctival PCR results. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Patient demographics and clinical characteristics

There were 157 COVID-19 patients from two centers included in this study. Demographic data and COVID-19 clinical characteristics are listed in Table 1. Seven non-COVID-19 patients (4.4%) and 30 discharged COVID-19 patients (19.1%) were merged into the non-COVID-19 group (23.6%), as they did not have any symptoms and had negative PCR tests at the time of the study. Suspected

COVID-19 patients were classified as such (50/157, 31.8%). Confirmed COVID-19 patients (70/157, 44.5%) were reclassified further into groups based on their symptoms. The mild COVID-19 group (57/157, 36.3%) consisted of patients with mild symptoms because there were no asymptomatic patients. The moderate-severe COVID-19 group (13/157, 8.3%) consisted of patients with either moderate (12/157, 7.6%) or severe symptoms (1/157, 0.6%). The latter group was from RSUI (13/157, 8.3%), while the other patients were from RSCM (144/157, 91.7%). The male-to-female ratio was approximately 2:3, with a median age of 33 years, and there were no differences across all the patient groups.

The symptoms of all subjects were listed chronologically based on their frequency in Table 1. The most dominant systemic symptoms that we observed were cough (55/157, 35%), fever (45/157, 28.7%), sore throat (30/157, 19.1%), hyposmia/anosmia (23/157, 14.6%), headache (20/157, 12.7%), and rhinorrhea (19/157, 12.1%). The SARS-CoV-2 PCR test was performed on NO and conjunctival swab samples for all patients. Thirteen patients were positive and classified as having confirmed COVID-19 with moderate and severe symptoms. Nineteen of 57 (33.3%) patients were positive and had mild symptoms. The remaining 38/57 (66.7%) patients with similar mild symptoms had negative results but had a previous positive result from another health center. Overall, a positive PCR test based on the conjunctival swab samples was obtained for 7/157 (4.5%) patients. A positive result was obtained for six COVID-19 patients (6/57, 10.5%) with mild symptoms and one non-COVID-19 patient (1/37, 2.7%). No patient had conjunctivitis symptoms. According to the Pango lineage, two samples from a conjunctival swab were submitted to GISAID and classified as B.1 and B.1.1.398. B.1 was the dominant lineage in all countries affected by the COVID-19 pandemic at that time [25] and this sublineage has been dominant in Indonesia. Moreover, B.1.1398 was an Indonesian-specific mutation that contributed to 5% of samples until June 2021 [26].

3.2. Dry eye subjective scores (OSDI) and objective examination results

We found that in all subjects, the OSDI score (median; IQR) decreased as follows: 2.3; 0–8 on D0, 0; 0–3 on D7, and 0; 0-0 on D14, which was statistically significant, D0 vs D7 p value < 0.001, D0 vs D14 p value < 0.0001, D7 vs D14 p value < 0.05 (Fig. 1). There were no differences in the OSDI scores between male and female subjects. The OSDI score tended to be higher in older subjects, with a Pearson correlation of 0.267 on D0, 0.326 on D7, and 0.219 on D14 (p value < 0.05) (Supplementary Table 1).

After analyzing the OSDI scores in each group, we found that only the moderate-severe COVID-19 group had OSDI scores greater than 13 or above the normal value on D0 (15.6; 9–28). Significant differences were found in the OSDI values between the moderate-

Table 1
Demographic data and COVID-19 related clinical characteristics.

	Total	No-COVID-19	Suspected COVID-19	Mild COVID-19	Moderate-Severe COVID-19	*P*-Value[a]
	N: 157	N: 37/157 (23.6%)	N: 50/157 (31.8%)	N: 57/157 (36.3%)	N: 13/157 (8.3%)	
Gender						
Male	62/157 (39.5%)	13/37 (35.1%)	18/50 (36%)	25/57 (43.9%)	6/13 (46.2%)	0,74
Female	95/157 (60.5%)	24/37 (64.9%)	32/50 (64%)	32/57 (56.1%)	7/13 (53.8%)	
Age (median, IQR)	33; 28-46	36; 28-46	32; 28-47	32; 28-43	46; 32-53	0,25
Symptoms						
Cough	55/157 (35%)	0/37 (0%)	23/50 (46%)	23/57 (40.4%)	9/13 (69.2%)	<0.001*
Fever	45/157 (28.7%)	0/37 (0%)	13/50 (26%)	20/57 (35.1%)	12/13 (92.3%)	<0.001*
Sore throat	30/157 (19.1%)	0/37 (0%)	12/50 (24%)	11/57 (19.3%)	7/13 (53.8%)	<0.001*
Headache	20/157 (12.7%)	0/37 (0%)	9/50 (18%)	8/57 (14%)	3/13 (23.1%)	0.046*
Rhinorrhea	19/157 (12.1%)	0/37 (0%)	8/50 (16%)	8/57 (14%)	3/13 (23.1%)	0.058
Myalgia	11/157 (7%)	0/37 (0%)	7/50 (14%)	4/57 (7%)	0/13 (0%)	0.057
Dyspnea	10/157 (6.4%)	0/37 (0%)	3/50 (6%)	3/57 (5.3%)	4/13 (30.8%)	0.001*
Ageusia	7/157 (4.5%)	0/37 (0%)	2/50 (4%)	4/57 (7%)	1/13 (7.7%)	0,40
Diarrhea	7/157 (4.5%)	0/7 (0%)	2/50 (4%)	3/57 (5.3%)	2/13 (15.4%)	0,13
Malaise	4157 (2.5%)	0/7 (0%)	1/50 (2%)	3/57 (5.3%)	0/13 (0%)	0,38
Nausea	2/157 (1.3%)	0/7 (0%)	1/50 (2%)	0/57 (0%)	1/13 (7.7%)	0,13
Cough	55/157 (35%)	0/37 (0%)	23/50 (46%)	23/57 (40.4%)	9/13 (69.2%)	<0.001*
Fever	45/157 (28.7%)	0/37 (0%)	13/50 (26%)	20/57 (35.1%)	12/13 (92.3%)	<0.001*
SARS-CoV-2 PCR test positive at						
Nasopharyngeal/oropharyngeal swab	32/157 (20.4%)	0/37 (0%)	0/50 (0%)	19/57 (33.3%)	13/13 (100%)	<0.001*
Conjunctival swab	7/157 (4.5%)	1/37 (2,7%)	0/50 (0%)	6/57 (10.5%)	0/13 (0%)	0.043*

severe COVID-19 group on D0 and the no COVID-19 group (0; 0–6, p value < 0.0001), suspected COVID-19 group (2.27; 0–9, p value < 0.001), and mild COVID-19 group (2.27; 0–7, p value < 0.001). A positive nasopharyngeal swab but not a positive conjunctival swab was associated with a higher OSDI value.

Table 2 presents the objective measurements for dry eyes, namely, the TMH, the NIKBUT, and ocular redness, based on the SARS-CoV-2 infection status. There were no differences found based on COVID-19 clinical classification, NO swab positivity, or conjunctival swab positivity. We also assessed the relationship between sex and age. Male subjects tended to have more ocular redness than female subjects (64.7% vs. 35.3%, p value < 0.05). A shorter NIKBUT and ocular redness were found more in subjects between the ages of 30 and 50 years old (56.9% p value < 0.05 and 70.6% p value < 0.05) (Supplementary Table 2).

3.3. Relationship between COVID-19 subjects and OSDI, TMH, and NIKBUT results

Fig. 2 depicts the differences in OSDI values in association with three objective measures of dry eye: the TMH, the NIKBUT, and ocular redness. We evaluated these data only in patients who underwent a Keratograph® examination because patients with severe COVID-19 were severely ill and unable to undergo the examination. Overall, there was no difference in OSDI values between those with no COVID-19 (0; 0–6) and suspected or mild COVID-19 (2.3; 0–7) (Fig. 2a). There was no significant difference in OSDI scores when we classified the categorical values of the TMH (normal 0; 0–4.5, low 2.3; 0–6.2, high 2.3; 0–11.3), the NIKBUT (normal 2.3; 0–4.5, short 0; 0–6.8) and ocular redness (normal 2.3; 0–5, redness 4.5; 0–11.3) (Fig. 2b–d).

We compared the OSDI values and objective measurement results between non-COVID-19 subjects and all COVID-19 patients (suspected and mild). In subjects with a high TMH, COVID-19 patients had a significantly higher OSDI score (5; 0–13.7) than non-COVID-19 patients (0; 0–4). Conversely, there were no significant differences in OSDI scores for patients with normal (non-COVID-19 (0; 0–6.8) vs. COVID-19 patients (2.3; 0–4.5)) and low TMH results (non-COVID-19 (1; 0–4) vs. COVID-19 patients (2.3; 0–6.8)) (Fig. 2e–g). In subjects with a short or very short NIKBUT, COVID-19 patients had a significantly higher OSDI score (2.3; 0–8) than non-COVID-19 patients (0; 0-0). In subjects with a normal NIKBUT, there was no significant difference in OSDI scores between non-COVID-19 (2.3; 0–8.5) and COVID-19 patients (2.3; 0–4.5) (Fig. 2h–i). There were no significant differences between the OSDI scores in subjects with no ocular redness (non-COVID-19 (0; 0–3) vs. COVID-19 patients (2.3; 0–6.8) or those with ocular redness (non-COVID-

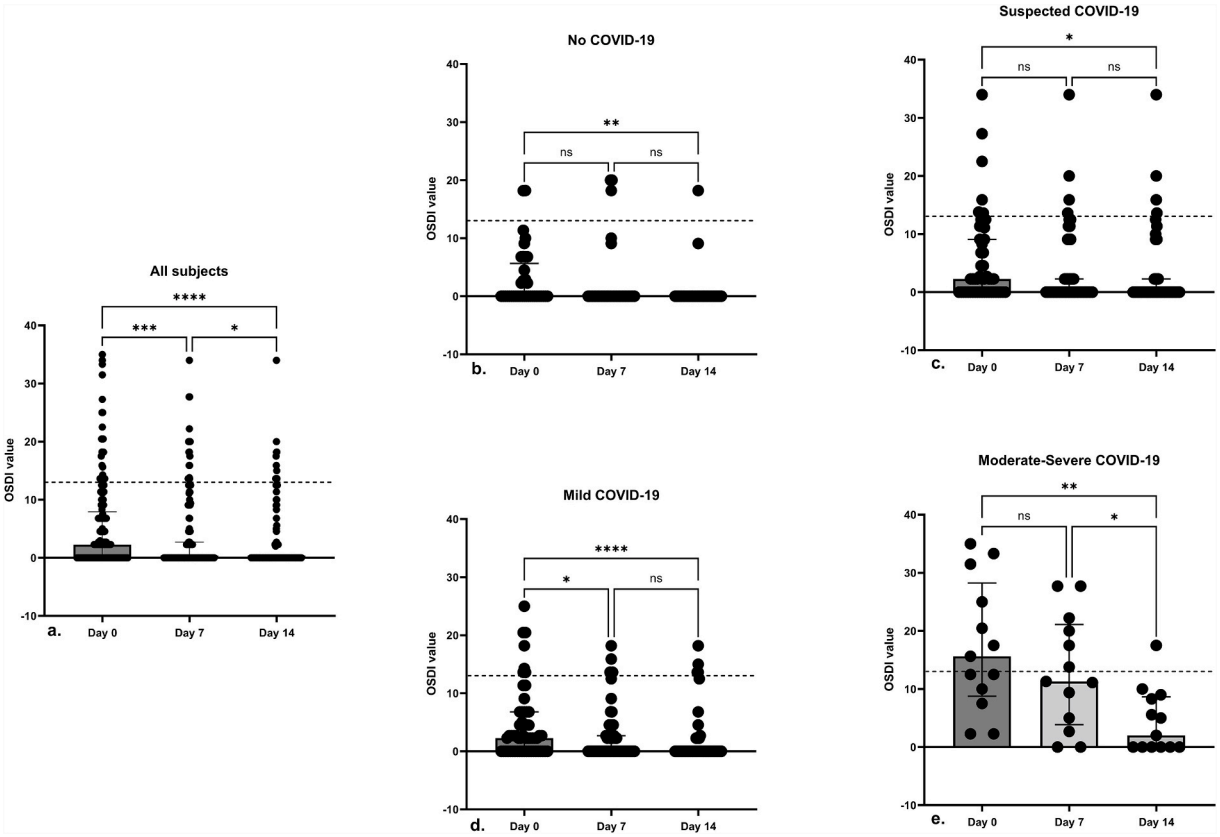

Fig. 1. (a–e). OSDI score distribution in each group per week (days 0, 7, and 14): OSDI score distribution in all subjects (a), OSDI score distribution in No COVID-19 group (b), OSDI score distribution in Suspected COVID-19 group (c), OSDI score distribution in Mild COVID-19 group (d), OSDI score distribution in Moderate-Severe COVID-19 group (e). Dash lines represent the cut-off value for normal OSDI. * p-value <0.05, ** p-value <0.01, *** p-value <0.001, **** p-value <0.0001.

Table 2
Dry eye subjective and objective measurement data.

	Total N: 157	OSDI (median; IQR) N = 157			TMH N = 140			NIKBUT N = 129		Redness N = 137	
		Day 0 N = 157	Day 7 N = 157	Day 14 N = 157	Normal N = 77	Low N = 36	High N = 27	Normal N = 57	Short N = 72	No Redness N = 120	Redness N = 17
Total		2.3; 0 - 8	0; 0 - 3	0; 0 - 0	77/140 (55%)	36/140 (26%)	27/140 (19%)	57/129 (44%)	72/129 (56%)	120/137 (88%)	17/137 (12%)
Gender											
Male	62/157 (39.5%)	2.3; 0 - 7	0; 0 - 2	0; 0 - 0	26/77 (33.8%)	19/36 (52.8%)	10/27 (37%)	21/57 (36.8%)	29/72 (40.3%)	43/120 (35.8%) *	11/17 (64.7%)*
Female	95/157 (60.5%)	2.3; 0 - 9	0; 0 - 5	0; 0 - 0	51/77 (66.2%)	17/36 (47.2%)	17/27 (63%)	36/57 (63.2%)	43/72 (59.7%)	77/120 (64.2%) *	6/17 (35.3%)*
Age											
(median; IQR) or Pearson Correlation	33; 28-46	r = 0.267**	r = 0.326**	r = 0.219**	32; 27 - 42	31.5; 29 - 36	35; 29-49	30; 28 - 44	33; 28 - 42	31; 28–41*	39; 33–50*
<30 years old	53/157 (33.8%)	2.3; 0 - 7	0; 0 - 2	0; 0 - 0	30/77 (39%)	8/36 (22.2%)	10/27 (37%)	25/57 (43.9%)*	21/72 (29.2%)*	47/120 (39.2%) *	1/17 (5.9%) *
30–50 years old	73/157 (46.5%)	2.3; 0 - 8	0; 0 - 4	0; 0 - 0	34/77 (44.2%)	21/36 (58.3%)	10/27 (37%)	20/57 (35.1%)*	41/72 (56.9%)*	53/120 (44.2%) *	12/17 (70.6%)*
>50 years old	31/157 (19.7%)	2.3; 0 - 14	0; 0 - 9	0; 0 - 5	13/77 (16.9%)	7/36 (19.4%)	7/27 (25.9%)	12/57 (21.1%)	10/72 (13.9%)	20/120 (16.7%)	4/17 (23.5%)
COVID-19 clinical classification											
No COVID-19	37 (23.6%)	0; 0 - 6	0; 0 - 0	0; 0 - 0	23/77 (29.9%)	8/36 (22.2%)	6/27 (22.2%)	16/57 (28.1%)	17/72 (23.6%)	32/120 (26.7%)	5/17 (29.4%)
Suspected COVID-19	50 (31.8%)	2.3; 0 - 9	0; 0 - 2	0; 0 - 2	22/77 (28.6%)	16/36 (44.4%)	9/27 (33.3%)	18/57 (31.6%)	26/72 (36.1%)	40/120 (33.3%)	5/17 (29.4%)
Mild COVID-19	57 (36.3%)	2.3; 0 - 7	0; 0 - 2	0; 0 - 0	32/77 (41.6%)	12/36 (33.3%)	12/27 (44.4%)	23/57 (40.4%)	29/72 (40.3%)	48/120 (40%)	7/17 (41.2%)
Moderate-Severe COVID-19	13 (8.3%)	15.6; 9–28***a	11.3; 4–21***a	2; 0–9**a	NA	NA	NA	NA	NA	NA	NA
Nasopharyngeal/oropharyngeal swab											
Negative	125/157 (79.6%)	2.3; 0 - 6	0; 0 - 2	0; 0 - 0	68/77 (88.3%)	30/36 (83.3%)	24/27 (88.9%)	50/57 (87.7%)	63/72 (87.5%)	105/120 (87.5%)	14/17 (82.4%)
Positive	32/157 (20.4%)	9.5; 2–18***	4; 0–14***	0; 0–8**	9/77 (11.7%)	6/36 (16.7%)	3/24 (11.1%)	7/57 (12.3%)	9/72 (12.5%)	15/120 (12.5%)	3/17 (17.6%)
Conjunctival Swab											
Negative	150/157 (95.5%)	2.3; 0 - 8	0; 0 - 3	0; 0 - 0	75/77 (97.4%)	33/36 (91.7%)	25/27 (92.6%)	54/57 (94.7%)	69/72 (95.8%)	115/120 (95.8%)	15/17 (88.2%)
Positive	7/157 (4.5%)	2.3; 0 - 3	0; 0 - 2	0; 0 - 2	2/77 (2.6%)	3/36 (8.3%)	2/27 (7.4%)	3/57 (5.3%)	3/72 (4.2%)	5/120 (4.2%)	2/17 (11.8%)

*p value < 0.05.
**p value < 0.01.
***p value < 0.001.
****p value < 0.0001.
[a] pairwise comparison to No COVID-19, adjusted to Bonferroni correction for multiple analysis.

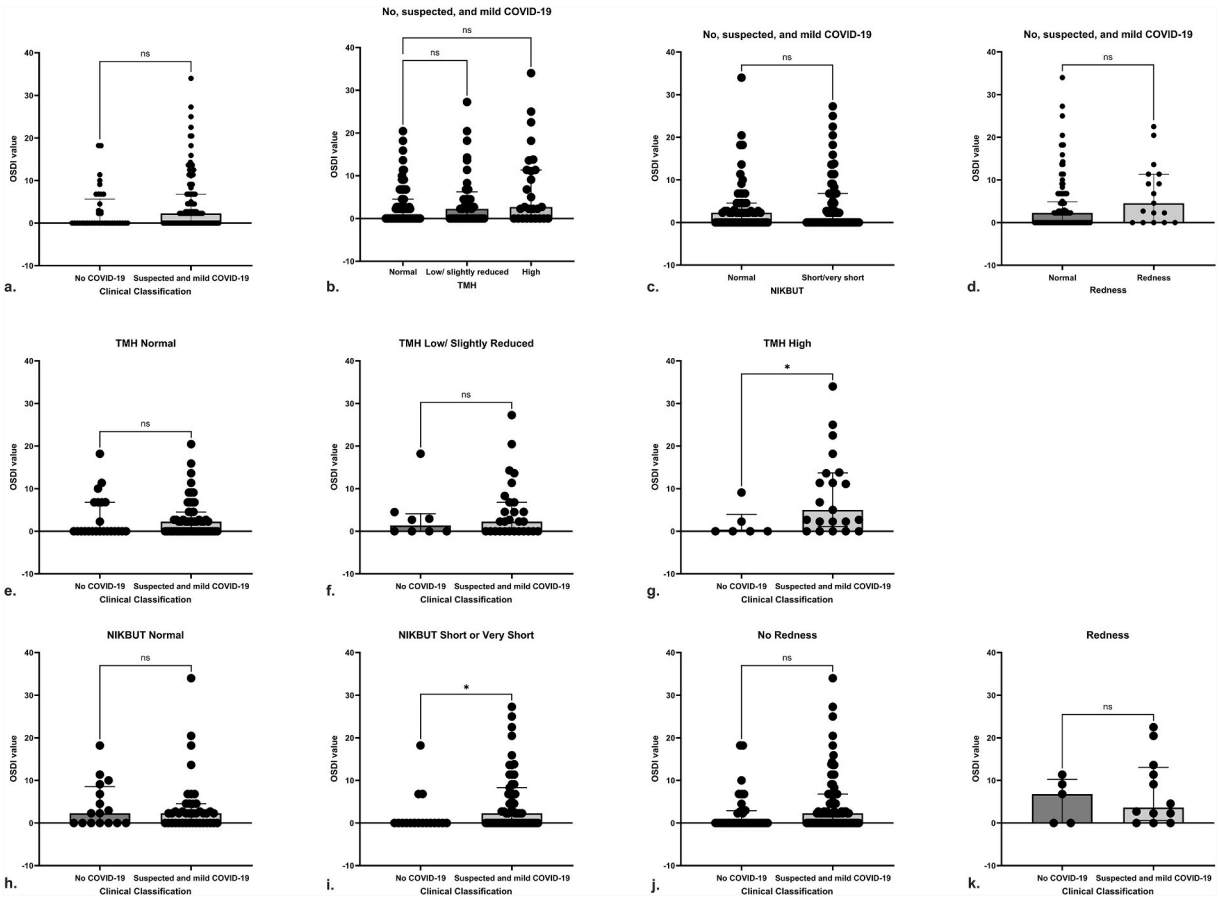

Fig. 2. (a–k). Differences in OSDI values in association with three objective measures of dry eye: TMH, NIKBUT, and ocular redness in the clinical classification group: OSDI values and COVID-19 status (No COVID-19 vs Suspected and mild COVID-19) (a), OSDI values and TMH measures in no, suspected, and mild COVID-19 patients (b), OSDI values and NIKBUT measures in no, suspected, and mild COVID-19 patients (c), OSDI values and redness in no, suspected, and mild COVID-19 patients (d), OSDI values and COVID-19 status in TMH normal group (e), OSDI values and COVID-19 status in TMH high group (f), OSDI values and COVID-19 status in TMH low/slightly reduced group (g), OSDI values and COVID-19 status in NIKBUT normal group (h), OSDI values and COVID-19 status in NIKBUT short/very short group (i), OSDI values and COVID-19 status in no ocular redness group (j), OSDI values and COVID-19 status in ocular redness group (k).

19 (6.8; 0–10) vs. COVID-19 patients (3.6; 0.5–13)) (Fig. 2j–k). Therefore, we did not find a significant correlation between dry eye symptoms and objective dry eye examination. However, COVID-19-related subjects have higher OSDI values in subjects with high TMH and short NIKBUT than the low/normal TMH and normal NIKBUT.

4. Discussion

Since 2019, the number of COVID-19 patients has grown and posed a tremendous threat to public health. SARS-CoV-2 was responsible for this current pandemic. Because of the virus' highly contagious nature, the role of the ocular surface as a possible portal of entry, reservoir for replication, and transmission of SARS-CoV-2 has been explored widely [27,28]. Ocular complaints in COVID-19 patients were also increasingly being reported even up to 40% of COVID-19 patients [11]. An implication for that, there may be a potential hazard to healthcare workers, including ophthalmologists who perform examination in close proximity to COVID-19 and exposed to tears.

Our research has unveiled a potential association between COVID-19 and dry eye symptoms (Supplementary Figure). Our study revealed that individuals with moderate-to-severe COVID-19 frequently reported subjective dry eye symptoms based on an OSDI questionnaire value greater than 13. After correlating the dry eye subjective complaints with three objective dry eye measurements, we found no correlation between the OSDI scores and the TMH, the NIKBUT, and ocular redness. Although we found a tendency in COVID patients where the symptoms were milder, more patients experienced red eyes, but that did not correlate clinically. However, we did not measure the same objective measurement with the Keratograph® in the moderate-severe group; thus, we could not compare the results among the groups. We noticed patients were also complaining of eye redness in the No-COVID group; therefore, eye redness was not a specific symptom for COVID-19. On the other hand, we discovered patterns that show that COVID-19 subjects with a high TMH

and a short NIKBUT displayed higher OSDI values than subjects with a low or normal TMH and a normal NIKBUT. These patterns might be related to the previous subjects' dry conditions, which our study could not determine.

This lack of association between dry eye symptoms and its objective parameters has been long known thus, dry eye is considered a symptomatic disease because the prevalence rates based on symptom reporting are more consistent than those based on signs [29]. As the subjective complaints occurred on the initial day of COVID-19 and were related to the severity of the disease, the OSDI questionnaire might be used at the beginning of the infection. Hong et al. found that the difference in OSDI scores before and after the onset of COVID-19 was statistically significant [30]. Our study also found that higher OSDI values were related to positive PCR tests for SARS-CoV-2 from NO swabs but not from conjunctival swabs, and the confirmatory test for COVID-19 is the positivity from NO swabs, not from the conjunctiva. Therefore, asking for dry eye symptoms could be used to ascertain the COVID-19 diagnosis. Further studies are necessary to confirm these findings in a larger population sample.

On the other hand, there is a hypothesis of the vulnerability of the dry eye population to COVID-19. Notably, the odds ratio of dry eye disease patients who contracted COVID-19 was 6.62 times higher than that of the general population [31]. The vulnerability of the dry eye population to infection has been argued to be due to the consequences of damage to the physical barrier on the ocular surface and a change in the composition of the tear film, which generally exhibits antimicrobial effects [32]. It was reported that SARS-CoV-2 infection correlated with small fiber neuropathy of the ocular surface, which can lead to dry eye symptoms [33]. Intriguingly, symptoms like dry eye disease, such as sore eyes, photophobia [34], ocular discomfort, discharge, and redness [35], have been reported in COVID-19 patients. Subjective eye complaints in a large study in China reported dry eye as one of the top three ocular symptoms of COVID-19 patients among 535 patients, in conjunction with blurred vision and foreign body sensation [36]. More cellular studies found that COVID-19 patients experienced ocular surface alterations, such as decreased goblet cell density and size and small fiber neuropathy in the cornea, with or without signs and symptoms of dry eye disease [33,37]. Unfortunately, a definitive understanding of why the OSDI score is different significantly only in a moderate-severe stage of COVID-19 remains unclear. Moreover, we could not perform and analyze the Keratograph® for patients in a moderate-severe group because all patients were hospitalized and needed intensive monitoring. We cannot determine the cause for the OSDI score difference between those groups at this stage.

Aside from dry eye symptoms, among all COVID-19 patients documented to date, ocular manifestations are present in between 5% and 11.6% of patients, and SARS-CoV-2 PCR results have been positive in one-third of ocular samples [35,38]. The ocular manifestations varied from conjunctivitis in between 2% and 30% of patients, conjunctival chemosis in 1% and 31% of patients, while ocular redness or conjunctival congestion was present in 1%–32% of patients [35]. In the literature, there are inconsistencies regarding whether ocular manifestations and evidence of SARS-CoV-2 in the eye correlate with the severity of COVID-19. Ritu et al. and Dutescu et al. revealed a higher detection rate of SARS-CoV-2 RNA in tears that reached 24–28% of patients with moderate to severe COVID-19 using three sampling methods (Schirmer and conjunctival swab, Schirmer test, and conjunctival swab) [27,39]. On the other hand, a similar study by Versery et al. and Furdova revealed that the RT−PCR test results for conjunctival sac swab samples from mechanically ventilated patients were much lower than those from nonmechanically ventilated patients (11%–12%) [40,41]. In our study, positive conjunctival swab samples were found in 7/157 (4.5%) subjects. On the contrary to other studies, SARS-CoV-2 RT-PCR was positive in one non-COVID-19 subject (1/37, 2.7%), six mild COVID-19 patients (6/57, 10.5%), and none in moderate-severe patients. This observation raises a critical point for consideration, highlighting the possibility of COVID-19 transmission via the ocular surface due to the unawareness of subjects with no or mild symptoms of COVID-19 and negative PCR test from the NO (Supplementary Figure).

The difference of ocular manifestations rate and positive SARS-CoV-2 RT PCR rate are probably due to the heterogeneity of the study populations across different research studies. The methods and techniques used for collecting ocular samples can vary between studies. In studies by Ritu et al. and Dutescu et al. used three different sampling methods, while Versery et al. and Furdova focused on conjunctival sac swabs. Differences in sampling methods and techniques can yield varying results in terms of viral detection rates. The timing of ocular sample collection in relation to the course of COVID-19 can be critical. Ocular manifestations and viral shedding may not be consistent throughout the entire disease course [27,39,40,42]. Our study found the presence of SARS-CoV-2 RNA shedding based on conjunctival swab assessment in seven patients (4% of all patients). Six patients had systemic symptoms, only two had ocular symptoms, and one had no symptoms. Interestingly, none of them were included in the moderate-severe COVID-19 group. Therefore, despite the primary mode of transmission of SARS-CoV-2 through respiratory droplets and contact with infected objects, another transmission mode, such as the ocular route, should not be underestimated, as the virus can be found in conjunctiva secretions and tears in patients with all levels of COVID-19 severity.

Ocular route might be important since several receptors (ACE2, TMPRSS2, ANPEP, DPP4, and NRP1) expressed on the ocular surface allow SARS-CoV-2 to replicate and act as a gateway for viral transfer to extraocular sites to establish a respiratory infection, making ocular surface cells a potential entry point and reservoir for transmission of the virus [9,43]. In addition, evidence from RT−PCR of SARS-CoV-2 in postmortem ocular tissue (including conjunctival tissue) suggests susceptibility of the ocular surface to SARS-CoV-2 infection [44]. Nevertheless, it has been suggested that the altered microbiota and microenvironment on the ocular surface could lead to pathogenic microbial overgrowth and local or systemic eye inflammation, which increases tear film instability [14].

Many studies have observed that after contracting COVID-19, patients still complain of COVID-19 symptoms, including ocular symptoms, that persist for a week or month, defined as "long COVID" or "post-COVID". Optic neuritis, retinal vascular occlusion, and uveitis have been reported in post-COVID-19 patients [45]. Moreover, COVID-19 patients with preexisting dry eye appeared to have worsened dry eye symptoms after the infection subsided, as evidenced by lower lipid layer thickness (LLT), worse papillae and meibom gland dysfunction (MGD), and a shorter NIKBUT [46]. A study by Brogna et al. hypothesized the underlying mechanism of long COVID-19, namely, that after the decrease in RNA SARS-CoV-2 viral load, it seems that the gut microbiome continues to produce toxin-like peptides that lead to bacterial dysbiosis [13,47]. This study is relevant, as many studies have highlighted the existence of a

gut-eye axis, where gut microbes can alter the immunity of the eye and contribute to dry eye [14,48]. Hence, we assumed that SARS-CoV-2 infection could cause, directly or indirectly, an inflammatory response and disturb the homeostasis of tear film composition, leading to dry eye and other ocular symptoms. Alternatively, coronaviruses have the properties of neurotropism and neuroinvasiveness, including sympathetic and parasympathetic nerve fibers in the lacrimal glands, leading to dry eye symptoms [12].

Our study had several limitations. First, we could not perform objective measurements of dry eye in the moderate-severe group due to hospital regulations and the severity of patients' conditions. Second, we did not differentiate subjects based on their disease onset, as we wanted to include all patients following the national criteria. Consequently, the number of subjects per group was not the same and was relatively small. Third, the brands of the kits we used to collect NO samples varied because we used the kits provided by the government based on existing stock. Also, we did not sequence the SARS-CoV-2 viruses in all samples therefore we could not compare symptoms between virus' variants. Additionally, we acknowledge the possible bias that dry eye symptoms may be caused by patients' activities during the pandemic, as patients spent more time in front of visual display terminals. This condition predisposes them to various health problems, including symptoms related to the ocular surface—i.e., dry eye, asthenopia (eye strain), eye fatigue, and sore eyes.

5. Conclusion

To conclude, our study underscores the intricate relationship between COVID-19 and dry eye, emphasizing the potential ocular surface manifestations. Dry eye symptoms were correlated with COVID-19 and that subjective eye complaints of dryness were more prevalent in the moderate-severe group. Subjective dry eye complaints were not associated with objective measurements such as the break-up time, the tear meniscus height, and redness. The presence of SARS-CoV-2 in conjunctival swabs, especially in asymptomatic and mild COVID-19 cases, denotes the possibility of ocular transmission due to the lack of awareness and the presence of SARS-CoV-2 receptors on the surface of the eye. Moreover, severe COVID-19 patients may be more susceptible to suffering from dry eye symptoms. Understanding the ocular implications of COVID-19 is crucial for comprehensive patient care and protecting the public as well as health workers to be more aware of COVID-19 transmission through tears or conjunctival secretions.

Ethics declaration

- This study was reviewed and approved by a joint ethics committee of the Faculty of Medicine, University of Indonesia–Cipto Mangunkusumo Hospital, with the reference number KET-727/UN2.FI/ETIK/PPM.00.02/2020 on July 13, 2020.
- All participants/patients (or their proxies/legal guardians) provided written informed consent to participate in the study.
- All participants/patients (or their proxies/legal guardians) provided written informed consent for the publication of their anonymised case details and images.

Data availability statement

The data underlying this study has not been deposited into a publicly available repository due to patients' privacy issue. The data are available from the corresponding author upon reasonable request. Due to subjects' privacy, the name will be provided in the form of an initial and the medical record number will not be included.

CRediT authorship contribution statement

Rina La Distia Nora: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Syaffa Sadida Zahra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Mei Riasanti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aliya Fatimah:** Writing – original draft, Project administration. **Rani Dwi Ningtias:** Writing – original draft, Project administration. **Fera Ibrahim:** Writing – review & editing, Supervision, Resources. **Budiman Bela:** Supervision, Resources. **R.R. Diah Handayani:** Supervision, Resources, Conceptualization. **Andi Yasmon:** Resources. **Made Susiyanti:** Writing – review & editing, Validation, Supervision. **Lukman Edwar:** Writing – review & editing, Validation, Supervision. **Yulia Aziza:** Writing – review & editing, Validation, Supervision. **Ratna Sitompul:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Rina La Distia Nora reports financial support was provided by Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan) with grant number RISPRO/MAN/COVID/B1/1/16/113/2020 and Design and Development Prototype Grant (Program Pendanaan Perancangan dan Pengembangan Purwarupa/P5) special assignment from Rector Universitas Indonesia with assignment number 722/SK/R/UI/2020. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28649.

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