INTRODUCTION

COVID-19 is the disease that caused the pandemic at the end of 2019, which was declared no longer an emergency. However, the number of new cases of COVID-19 with severe cases and deaths. The upper respiratory microbiota has been associated with susceptibility to viral infection and disease severities. However, the association of respiratory tract microbiota with the modulation of COVID-19 outcomes still raises a question. Therefore, we examined the association between the diversity of upper respiratory microbiota and clinical manifestations of COVID-19.

Methods: This research is an observational cross-sectional study. We characterized the microbiota in the upper airway using the 16S ribosomal RNA sequencing method in 74 COVID-19 patients aged 18-64 years. We also examined the association between COVID-19 severities with alpha and beta diversity of upper respiratory microbiota. The alpha and beta diversity was analyzed by QIIME and shown by R software, AMOVA and MRPP testing was done with R software. The t test and diagrams were performed using R software. Bivariate analysis was carried out by t-test and Chi-square test.

Results: The top five phyla in the upper respiratory tract of Indonesian COVID-19 patients with age 18-64 years old were Firmicutes (32,3%), Bacteroidia (27,1%), Fusobacteriota (15,2%), Proteobacteria (15,1%) and Actinobacteria (7,1%). Shannon and ACE index analysis showed no decline of microbiota diversity in the upper airway with increased disease severity. However, there were significant beta diversity differences in the upper airway microbiota between mild and severe COVID-19. The Firmicutes phylum and Orbitabacterium genus abundance were significantly higher in severe COVID-19 than in mild. The quantity of the Fusobacteriota phylum, Neisseria, and Fusobacterium genera was significantly higher in severe COVID-19 than in mild.

Conclusion: Our research supports the relationship between the severity of COVID-19 and the diversity of the microbiota in the upper airway in adults. Further studies are needed to examine how microbiota prevents COVID-19 severities.
Pathogenic viruses in the respiratory tract can change the microbiota in the respiratory tract, resulting in a high abundance of opportunistic bacteria that can exacerbate infectious diseases in patients. Therefore, characterization of the nasopharyngeal microbiota can be a predictor of the severity of infection in the respiratory tract in several diseases.

Most studies observed significant differences in the diversity of nasopharyngeal microbiota with disease severities of COVID-19. However, its association in Indonesian adults was not conducted yet. This study aimed to characterize and determine the association of microbiota diversity with disease severity in COVID-19 adult patients in Indonesia.

METHODS

Study design and subject recruitment
This research is an observational cross-sectional study. Seventy-four nasopharyngeal and oropharyngeal swab specimens were obtained from 18-64 years old patients suspected of COVID-19 from dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, and Sari Asih Ciledug Hospital, in the Western part of Java Island, April 2020 – April 2021. The study's inclusion criteria were patients clinically suspected of COVID-19 or had previous close contact with COVID-19 patients, registered from April 2020 – April 2021 in dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, and Sari Asih Ciledug Hospital, the sample age range from 18-64 years old. The exclusion criteria were incomplete data, and the sample refused to join this study. The nasopharyngeal and oropharyngeal swabs were collected based on CDC guidelines by a trained health provider. Swabs were collected immediately when patients were clinically suspected of COVID-19 or had a history of close contact with COVID-19 patients. The swab was put into the viral transport medium (VTM) and vortexed before aliquoting. Samples with clear and thick bands at OD260, and the quality of the DNA was determined by electrophoresis on an agarose gel. High molecular weight DNA without any stains or impurities in the gel indicates high-quality DNA. DNA in TE buffer was stored in a -20°C freezer before use.

**Bacterial 16S ribosomal RNA sequencing**
To determine how upper respiratory microbiota affected COVID-19 disease severities, a 16S rRNA gene amplicon sequencing method was used to compare mild and severe COVID-19. For taxonomic grouping, individual sequencing of highly variable areas was performed. Based on the pair-end algorithm, the Illumina HiSeq platform was used for amplicon sequencing. Seventy-four DNA in TE buffer was sent to Genetica Science Laboratory for bacterial 16S ribosomal RNA sequencing. Until the final sequencing data is obtained, the DNA goes through several stages of examination, PCR amplification, purification of PCR products, preparation of database (library) and sequencing (HiSeq). The 16S rRNA/18SrRNA/ITS genes from unique areas were amplified using primers with barcodes. The Phusion High-Fidelity PCR Master Mix® (Thermo Fisher Scientific, Greenville, NC) was used for the PCR test. Then the PCR product was mixed with 1x loading buffer (including SYB green) with the same amount, and electrophoresis was carried out on 2% agarose gel for visualization. Samples with clear and thick bands at 400-450 bp were chosen for the next examination. Then, the PCR product was purified with Qiagen Gel Extraction Kit® (Qiagen, Hilden, Germany). Data libraries created with TruSeq DNA PCR-Free Sample Preparation Kit® (Illumina, USA) and quantified with Qubit and Q-PCR were analyzed with HiSeq2500 PE250. Quality control (QC) is carried out at every stage of the method to maintain data reliability.

**Analysis of Sequencing Results**
Amplicons were sequenced with Illumina HiSeq's paired-end program to produce 250 bp pair-end raw reads (Raw PE). Paired-end readings are transferred to samples using unique barcodes and are reduced by cutting the barcodes and primer sequences. FLASH then connects the paired-end readings. (v1.2.7, http://ccb.jhu.edu/software/FLASH) to achieve high-quality Clean Tags based on the Qiime quality control procedure (v1.7.0, http://qiime.org/scripts/split_libraries_fasta.html). Tags are compared to a reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using the UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html), to identify chimera sequences in Clean Tags (http://www.drive5.com/usearch/manual/chimera Formation.html). And then, the chimera sequences are discarded to get the last Effective Tags. The sequence evaluation used all effective tags with Uparse software (Uparse v7.0.1001 http://drive5.com/uparse/). To analyze species diversity in each sample, all Effective Tags are grouped based on 97% similarity of DNA sequences into OTUs (Operational Taxonomic Units). Subsequent analysis was performed by selecting representative sequences for every OTU. For every usual sequence, the mothur program was run against the SSUrRNA database from the SILVA database (http://www.arb-silva.de/) for species analysis at every taxonomical level (threshold: 0.8~1) (kingdom, phylum, class, order, family, genus, species). The MUSCLE (version 3.8.31, http://www.drive5.com/muscle/) can assess numerous sequences quickly to attain phylogenetic relationships of all typical sequences. Most OTU information is normalized to the number of standardized sequences corresponding to the sample with minimal sequences.
**Statistical Analysis**

Sequencing data were further analyzed for phyla and genera distribution, alpha diversity, and beta diversity, which is done based on the normalized data output. Alpha diversity was applied by biodiversity complexity analysis for each sample through the Shannon and ACE index, which was analyzed by QIIME (Version 1.7.0) and shown by R software (Version 2.15.3). Beta diversity analysis was managed to calculate sample variations in species density. AMOVA and MRPP testing was done with R software (Vegan package: MRPP function). The t test and diagrams were performed using R software. Bivariate analysis to determine whether the various levels of taxa including phyla and genera, varied significantly between severe and mild COVID-19, was carried out by t-test. Bivariate analysis was performed using the Chi-square test to verify whether the presence of the upper respiratory pathogen of aerobic and anaerobic bacteria significantly differed among severe and mild COVID-19. The significant p-value is <0.05

**Determination of the severity of COVID-19**

COVID-19 severity was defined by the hospital doctor in charge of the patient based on the WHO 2020 guidance (1). We divided 74 patients into two groups, 37 mild (asymptomatic and mild) and 37 severe (moderate, severe, and critical) groups, based on clinical and laboratory data at the end of COVID-19 treatment or 7 days or more after COVID-19 confirmation.

The ethics committee of the Faculty of Medicine at Universitas Indonesia accepted this study (KET-1436/UN2. F1/ETIK/PPM.00.02/2020). All patients involved in this study had given informed consent.

**RESULTS**

**Patient’s characteristics**

From patient characteristics, there was no difference in gender between mild and severe COVID-19 patients (p = 1.000). However, older patients tend to have severe diseases. In severe COVID-19, there were more patients with fever, cough, shortness of breath, weakness, and sore throat (p < 0.05) (Table 1). No difference in rhinorrhea (p = 0.398) and anosmia (p = 1.000) symptoms between severe and mild COVID-19 (Table 1).

**Characteristics of Microbiota in COVID-19 Patients in Indonesia**

The Most Abundant Phyla and Genera Microbiota analysis showed that the five most abundant phyla from the nasopharynx and oropharynx of COVID-19 patients were Firmicutes (32.3%), Bacteroidota (27.1%), Fusobacteriota (15.2%), Proteobacteria (15.1%), and Actinobacteria (7.1%) (Figure 1).

While the five most abundant genera from the nasopharynx and oropharynx of COVID-19 patients are Prevotella (20.5%), Streptococcus (20.5%), Leptotrichia (7.9%), Neisseria (7.0%), and Fusobacterium (6.8%) (Figure 2).

**Microbiota Profile in Severe and Mild COVID-19**

There is no difference in alpha diversity in the nasopharyngeal and oropharyngeal microbiota, the microbial diversity within each group of mild and severe COVID-19 patients, as calculated by the Shannon (p = 0.117) and ACE index (p = 0.445) (Figure 3).

**Table 1. Characteristics and severity of COVID-19 patients.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Severe COVID-19 (n=37) (n, %)</th>
<th>Mild COVID-19 (n=37) (n, %)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)†</td>
<td>43 (21-65)</td>
<td>31 (19-43)</td>
<td>0.020*</td>
</tr>
<tr>
<td>Male</td>
<td>15 (41)</td>
<td>15 (41)</td>
<td>1.000</td>
</tr>
<tr>
<td>Fever or history of fever</td>
<td>27 (73)</td>
<td>2 (5)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>10 (27)</td>
<td>6 (16)</td>
<td>0.398</td>
</tr>
<tr>
<td>Cough</td>
<td>31 (84)</td>
<td>11 (30)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>20 (54)</td>
<td>4 (11)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Anosmia</td>
<td>3 (8)</td>
<td>2 (5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Fatigue</td>
<td>22 (60)</td>
<td>4 (11)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Sore throat</td>
<td>14 (38)</td>
<td>3 (8)</td>
<td>0.005**</td>
</tr>
<tr>
<td>Antibiotics usage</td>
<td>34 (92)</td>
<td>12 (32)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Comorbidty</td>
<td>14 (38)</td>
<td>1 (3)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Note: † Median, range; *Significant by Mann-Whitney U test; **Significant by Chi-square test.
Prevotella aurantica, Prevotella loescheii, Prevotella baroniae, Prevotella oralis, Prevotella micans, and Bacteroides fragilis as possible lower respiratory pathogen bacteria were found as normal flora in URT.

Focusing in Prevotella buccae (p = 0.005) and Prevotella disiens (p = 0.043) were significantly lower in severe COVID-19 compared to mild.

DISCUSSION

The microbiota in the respiratory tract significantly assists the host in fighting bacterial and viral infections when pathogens enter the upper respiratory tract. The nasopharyngeal microbiota also regulates adaptive immune responses. Some bacteria can inhibit viral infections by producing proinflammatory cytokines stimulated by the bacteria or their components. To assess the relationship between microbiome and COVID-19 severity, we examined the bacteria in the upper respiratory tracts of Indonesian patients.

Our study results showed that the median age in patients with severe COVID-19 was higher than mild. This is in line with the report from Surendra et al. (2021) that a higher risk of death is associated with older age. Anosmia and rhinorrhea symptoms are not significantly different between severe and mild COVID-19. Anosmia and rhinorrhea were related to COVID-19 infection, but their relationship with severity was not defined yet. Our study showed no difference in alpha diversity in the nasopharyngeal and oropharyngeal microbiota in the severe and mild COVID-19 patient groups. Similar results were found in the previous study. However, Bose et al. (2022) showed alpha diversity decreased in COVID-19 patients compared to non-COVID-19, depending on the COVID-19 wave.

There were significant differences in beta diversity in the nasopharyngeal and oropharyngeal microbiota between the groups of mild and severe COVID-19 patients, as determined by AMOVA (p = 0.032) and MRPP analysis (p = 0.034).

The top ten most abundant phyla of severe and mild COVID-19 groups in this study can be seen in Figure 4. Statistical analysis of all phyla in this study showed that the abundance of Firmicutes phyla was significantly more profound in severe COVID-19 than in mild (p = 0.014). Meanwhile, the quantity of the Fusobacteria phyla was significantly lower in severe COVID-19 compared to mild (p = 0.026) (Figure 5).

The top ten most abundant genera of severe and mild COVID-19 groups in this study can be seen in Figure 6. Statistical analysis of all genera showed that Neisseria (p-value of 0.037) and Fusobacterium (p-value of 0.033) were lower in severe COVID-19 than in mild and Oribacterium (p-value of 0.035) was higher in mild COVID-19 than in severe (Figure 5).

Characteristics of Pathogenic Bacteria

We didn’t find any upper respiratory tract pathogenic bacteria in this study. However, several bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Sphingomonas paucimobilis, Acinetobacter haemolyticus, Acinetobacter junii, Prevotella intermedia, B. The microbiota in the respiratory tract significantly assists the host in fighting bacterial and viral infections when pathogens enter the upper respiratory tract. The nasopharyngeal microbiota also regulates adaptive immune responses. Some bacteria can inhibit viral infections by producing proinflammatory cytokines stimulated by the bacteria or their components. To assess the relationship between microbiome and COVID-19 severity, we examined the bacteria in the upper respiratory tracts of Indonesian patients.

Our study results showed that the median age in patients with severe COVID-19 was higher than mild. This is in line with the report from Surendra et al. (2021) that a higher risk of death is associated with older age. Anosmia and rhinorrhea symptoms are not significantly different between severe and mild COVID-19. Anosmia and rhinorrhea were related to COVID-19 infection, but their relationship with severity was not defined yet.

Our study showed no difference in alpha diversity in the nasopharyngeal and oropharyngeal microbiota in the severe and mild COVID-19 patient groups. Similar results were found in the previous study. However, Bose et al. (2022) showed alpha diversity decreased in COVID-19 patients compared to non-COVID-19, depending on the COVID-19 wave.

There were significant differences in beta diversity in the nasopharyngeal and oropharyngeal microbiota between the groups of mild and severe COVID-19 patients, as determined by AMOVA (p = 0.032) and MRPP analysis (p = 0.034).

The top ten most abundant phyla of severe and mild COVID-19 groups in this study can be seen in Figure 4. Statistical analysis of all phyla in this study showed that the abundance of Firmicutes phyla was significantly more profound in severe COVID-19 than in mild (p = 0.014). Meanwhile, the quantity of the Fusobacteria phyla was significantly lower in severe COVID-19 compared to mild (p = 0.026) (Figure 5).

The top ten most abundant genera of severe and mild COVID-19 groups in this study can be seen in Figure 6. Statistical analysis of all genera showed that Neisseria (p-value of 0.037) and Fusobacterium (p-value of 0.033) were lower in severe COVID-19 than in mild and Oribacterium (p-value of 0.035) was higher in mild COVID-19 than in severe (Figure 5).

Characteristics of Pathogenic Bacteria

We didn’t find any upper respiratory tract pathogenic bacteria in this study. However, several bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Sphingomonas paucimobilis, Acinetobacter haemolyticus, Acinetobacter junii, Prevotella intermedia, Prevotella baroniae, Prevotella oralis, Prevotella micans, and Bacteroides fragilis as possible lower respiratory pathogen bacteria were found as normal flora in URT. Focusing in Prevotella buccae (p = 0.005) and Prevotella disiens (p = 0.043) were significantly lower in severe COVID-19 compared to mild.

DISCUSSION

The microbiota in the respiratory tract significantly assists the host in fighting bacterial and viral infections when pathogens enter the upper respiratory tract. The nasopharyngeal microbiota also regulates adaptive immune responses. Some bacteria can inhibit viral infections by producing proinflammatory cytokines stimulated by the bacteria or their components. To assess the relationship between microbiome and COVID-19 severity, we examined the bacteria in the upper respiratory tracts of Indonesian patients.

Our study results showed that the median age in patients with severe COVID-19 was higher than mild. This is in line with the report from Surendra et al. (2021) that a higher risk of death is associated with older age. Anosmia and rhinorrhea symptoms are not significantly different between severe and mild COVID-19. Anosmia and rhinorrhea were related to COVID-19 infection, but their relationship with severity was not defined yet.

Our study showed no difference in alpha diversity in the nasopharyngeal and oropharyngeal microbiota in the severe and mild COVID-19 patient groups. Similar results were found in the previous study. However, Bose et al. (2022) showed alpha diversity decreased in COVID-19 patients compared to non-COVID-19, depending on the COVID-19 wave.

There were significant differences in beta diversity in the nasopharyngeal and oropharyngeal microbiota between the groups of mild and severe COVID-19 patients, as determined by AMOVA (p = 0.032) and MRPP analysis (p = 0.034).

The top ten most abundant phyla of severe and mild COVID-19 groups in this study can be seen in Figure 4. Statistical analysis of all phyla in this study showed that the abundance of Firmicutes phyla was significantly more profound in severe COVID-19 than in mild (p = 0.014). Meanwhile, the quantity of the Fusobacteria phyla was significantly lower in severe COVID-19 compared to mild (p = 0.026) (Figure 5).

The top ten most abundant genera of severe and mild COVID-19 groups in this study can be seen in Figure 6. Statistical analysis of all genera showed that Neisseria (p-value of 0.037) and Fusobacterium (p-value of 0.033) were lower in severe COVID-19 than in mild and Oribacterium (p-value of 0.035) was higher in mild COVID-19 than in severe (Figure 5).

Characteristics of Pathogenic Bacteria

We didn’t find any upper respiratory tract pathogenic bacteria in this study. However, several bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Sphingomonas paucimobilis, Acinetobacter haemolyticus, Acinetobacter junii, Prevotella intermedia, Prevotella baroniae, Prevotella oralis, Prevotella micans, and Bacteroides fragilis as possible lower respiratory pathogen bacteria were found as normal flora in URT. Focusing in Prevotella buccae (p = 0.005) and Prevotella disiens (p = 0.043) were significantly lower in severe COVID-19 compared to mild.
Proteobacteria and Actinobacteria were the five most abundant phyla in COVID-19 patients in our study. Similar to the survey by Kolhe et al. in Marseille, France\(^\text{15}\), Qin et al. in Beijing, China\(^\text{7}\) and Ventero et al. in Alicante, Spain\(^\text{6}\) It is indicated that geographical area doesn't contribute to the top five phyla abundances in COVID-19 patients. Therefore, we continued to analyze within genera abundance, and we found there are differences, where: Prevotella, Streptococcus, Fusobacterium, Leptotrichia, and Neisseria were the five most abundance genera in COVID-19 patients in our study, similar to Ventero et al. in top two genera.\(^\text{6}\) Meanwhile, Staphylococcus, Corynebacterium, and Dolosigranulum were the most abundant genera in Nashville, Tennessee and Bronx, New York.\(^\text{16}\)

We identified that the abundance of Firmicutes phylum was significantly higher in severe COVID-19 than in mild COVID-19, while the abundance of Fusobacteriota phylum was inverted. Kolhe et al. (2021) reported abundant Cyanobacterial taxa at the phylum level in asymptomatic and symptomatic COVID-19 patients.\(^\text{15}\) We found that the abundance of Neisseria and Fusobacterium was significantly lower in severe COVID-19; Oribacterium was significantly higher in severe. Meanwhile, other studies report the abundance of different genera.\(^\text{6,7,15,16}\)

Leptotrichia, Treponema, Gemella, Parvimonas, Alloprevotella, Lachnanaerobaculum, Porphyromonas, and Streptococcus can differentiate mild COVID-19 from severe, supporting their potential use as biomarkers.\(^\text{13}\) The nasal microbiome of symptomatic COVID-19 patients was significantly enhanced with Cutibacterium and Lentimonas. A significantly lower abundance of five bacterial taxa was also obtained: Prevotellaceae, Luminiphilus, Flectobacillus, Comamonas, and Jannaschia in symptomatic COVID-19 patients. Community disturbance of the nasal microbiota in COVID-19 may play a role in influencing the severity of COVID-19.\(^\text{15}\)

The potential pathogen anaerobe bacteria Prevotella buccal and Prevotella disiens presence differed significantly lower in severe COVID-19 compared to mild in our study. Xiong et al. report a significantly declined species abundance of the Prevotella genus in COVID-19 samples.\(^\text{15}\) In the respiratory tract, research reports the probable contribution of oral bacteria, including some Prevotella organisms, in long COVID-19.\(^\text{18}\) Gauthier et al. report the abundance of opportunistic pathogens such as the genera Actinomyces, Prevotella, Rothia, Streptococcus and Veillonella was lower in severe COVID-19.\(^\text{13}\)

The difference in the abundance of genera between the several studies could be because of the age discrepancies across the research samples. The age
of the patients in this study was in the adult range. The characteristic of the nasopharyngeal microbiota varies with age. The older generation is linked with decreasing Moraxella and Dolosigranulum abundances and increasing Corynebacterium and Staphylococcus. Lawsonella and Peptostreptococcus were more prevalent in study samples aged 12 years or more but were detected in 21% and 19% of kids aged 8 years or less. The diversity and composition of the nasopharyngeal microbiome are convincingly related to age and age changes the relationship between some bacterial taxa and SARS-CoV-2 infection level.  

Differences in phylum and genera abundance also occur in COVID-19 patients with and without comorbidities. In our study, COVID-19 patients with comorbid (hypertension, obesity, diabetes mellitus, COPD, cancer, CKD, or CAD) have a higher abundance of Firmicutes, Actinobacteria, and Proteobacteria phyla, whereas the abundance of these phyla and genera were less abundant in those without comorbidity. The study by Kim et al. (2022) reported that administering Azithromycin and other classes of antibiotics before nasopharyngeal and oropharyngeal swab specimens’ collection, initially thought to cause differences in the results of microbiota composition analysis in the upper respiratory tract. Whether the period between giving antibiotics and obtaining nasopharyngeal and oropharyngeal swabs (in this study, between 13 days before to 13 days after swab collection) affects the differences in the abundance of these phyla and genera can't be concluded. However, the DNA-based sequencing test, in which the DNA of bacteria that have died from antibiotics within 0-6 months did not affect the sequencing results of the upper respiratory tract microbiota. Korkmaz et al. (2022) report that administering antibiotics within 0-6 months did not cause disturbances in the composition of the upper respiratory tract flora.  

Our study is cross-sectional data only retrieved at a time. Thus we can only know about the relationship and its characteristic. Further case-control studies will be able to show whether SAR-CoV-2 infection causes dysbiosis of the microbiota in the upper airway or the different composition of the microbiota a person’s upper respiratory tract before being exposed to COVID-19 which causes differences in the severity of the clinical manifestations of COVID-19 and play a part in the development of bacterial coinfections during COVID-19. So, modulation of upper airway microbiota composition due to dysbiosis can be an alternative to preventing severe COVID-19.  

CONCLUSION  

In summary, our findings show a link between the severity of SARS-CoV-2 infection and microbiota features in the upper respiratory tract of adults. Further research with more sample extents and case-control design is required to observe how the interaction between SARS-CoV-2 and the bacterial community can prevent the severity of clinical manifestations and the recovery of COVID-19 patients.

ACKNOWLEDGEMENTS  

We want to present our greatest gratitude to the Head of the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia, Pratiwi Sudarmono and Fera Ibrahim; Director of Dr. Cipto Mangunkusumo National Central General Hospital, Lies Dina Liastuti; Director of Sari Asih Ciledug Hospital, Ni'matullah Mansur; Development Director of Sari Asih Group Hospital, Aditya Marliana Bintari; and all laboratory and hospital staff for compassion and provision in our research.
REFERENCES


