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# A Community's Knowledge and Attitude in Recognizing Symptoms and Diarrhea Management in Children

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***Abstract***

***Background****: Diarrhea is the world's second-biggest cause of death among children. Children suffer from severe dehydration due to the lack of understanding of treating diarrhea properly.* ***Objective****: This study aimed to assess the level of community knowledge and attitudes toward the treatment of diarrhea in children.* ***Methods****: This was an observational, cross-sectional study conducted in a community in Gresik, Indonesia, in 2019. A Likert scale questionnaire, consisting of 10 items on knowledge and five on community attitudes on diarrhea management in children, was used to collect data.* ***Results****: One hundred and seven participants responded to the survey, and 89.72% were women. 'Respondents' mean age was 41.84 ± 11.27 years with various levels of education. It was found that the community had good knowledge of diarrhea management in children, as shown by the finding that 84.11% of the participants provided the correct answers. About 87.85% of the participants were aware of the signs and symptoms of diarrhea in children. However, there is still a shortage of understanding about managing diarrhea properly, particularly when identifying the necessary medicine. About 89.72% of the participants demonstrated a "positive" attitude in diarrhea management, while approximately 78.51% agreed that children with diarrhea should be given oral rehydration.* ***Conclusion****: The knowledge about recognizing signs and symptoms of diarrhea in children was good, while the knowledge about diarrhea management needed to be improved. Overall, the respondents had a positive attitude about diarrhea management in children.*

***Keywords****: attitude, children, diarrhoea, knowledge, medication use*

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## INTRODUCTION

Diarrhea is a typical symptom of gastrointestinal infections caused by bacteria, viruses, and protozoa (WHO, 2017). It is defined as loose or liquid feces occurring at least three times per day or more frequently than the individual's regular frequency (UNICEF & WHO, 2009).

Diarrhea is the second-biggest cause of death in children globally, claiming the lives of 525,000 children under the age of five each year (UNICEF & WHO, 2013; WHO, 2017). Based on UNICEF's data in 2017, about 6% of Indonesian children's deaths under five years are caused by diarrhea (UNICEF, 2018). Diarrhea in children is a serious concern because it could result in malnutrition, electrolyte imbalance, and death because of dehydration (Cotran *et al*., 1999).

Dehydration in diarrhea occurs when there is an insufficient replenishment of water and electrolytes (sodium, chloride, potassium, and bicarbonate) in the stool, thereby causing a deficit of water and electrolytes (WHO, 2005; Cajacob & Cohen, 2016). Dehydration can be preventable by providing an oral rehydration solution to the patient; this simple method can be used at home and prevents death caused by dehydration by more than 90% (WHO, 2005).

Mothers' knowledge is a crucial factor in children's diarrheal dehydration (Christy, 2014). Improved community knowledge about diarrhea in children is one approach that can help to prevent child mortality from diarrhea (UNICEF & WHO, 2009). In developing countries, a lack of information about diarrhea in children leads to inadequate prevention and management (Bhatnagar *et al*., 2010). Mothers with sufficient diarrhea knowledge can prevent and protect their children from dehydration, malnutrition, and mortality due to diarrhea (Sulisnadewi *et al*., 2012). Improved knowledge on how to treat diarrhea in children may help to reduce morbidity and mortality (Rehan *et al*., 2003). Therefore, the objective of this study was to examine community knowledge and attitudes toward symptoms recognition and the use of medications in the treatment of diarrhea in children. In addition, the relationship between sociodemographic characteristics and community knowledge and attitude was investigated.

## MATERIALS AND METHODS

### Materials

Participants who filled the informed consent were asked to fill out the questionnaires. The questionnaires had three sections:

1. Socio-demographics: age, gender, level of education, occupation, and diarrhea history of their children.
2. Knowledge of diarrhea in children: which consisted of ten questions on a 3-point Likert scale about the knowledge of signs, symptoms, treatment, and the use of medication for diarrhea in children. One point was given for 'do not know,' two points for a 'false' answer, and three points for a 'true' answer. The scores ranged from 33% to 100%, the participants were categorized as 'good' if they had scored above 66.67%, and 'poor' if they had a score of less than 66.67%.
3. Attitude toward managing diarrhea in children: five statements on a 5-point Likert Scale were given for the attitudes toward managing diarrhea in children. The scores ranged from 1 point for 'strongly disagree' to 5 points for 'strongly agree. ‘The scores were 20% to 100%, and participants were categorized as 'positive' if they scored above 60.00%.

Prior to the distribution of the questionnaires, validity and reliability testing were performed. The questionnaires' validity was examined using Spearman's method, and the results were confirmed to be valid (significance level 5%). After testing using Cronbach's alpha, the questionnaires exhibited high reliability for knowledge (0.787) and attitude (0.759).

### Method

This observational, cross-sectional study was conducted in a community in Gresik, Indonesia, in 2019. The Health Research Ethics Committee at the Faculty of Public Health, Universitas Airlangga, Indonesia, authorized the study's protocol with the number of ethical approval of 217/EA/KEPK/2020. This study used purposive sampling. The sample size was calculated using Slovin's formula with a minimum sample of 83 participants. Participants included in this study were parents who could read, write, and communicate in Indonesian and had at least one child minimally aged 18 years old. The participants received information sheets about this study and had no intervention from researchers to fill in the questionnaires.

SPSS was used to analyze the data received from the questionnaires. Sociodemographic data were then analyzed using descriptive statistics. Finally, a Spearman correlation test was employed to assess the association between variables, with a p-value of 0.05 was regarded as statistically significant.

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## RESULTS AND DISCUSSION

The validity of the questionnaire was verified on 30 respondents before it was used in the survey. Cronbach's alpha was used to examine the reliability tests after the validity score was acquired using Spearman's method. This study's questionnaire was confirmed to be statistically valid (significance level < 5%) and had 'high' reliability for knowledge (0.787) and attitude (0.759).

One hundred and seven participants gave their consent to participate in the survey. The characteristics are presented in Table 1. Females made up 89.72% of the respondents. Their ages varied from 17 to 70 years old (with a mean age of 41.84 + 11.27). Most of the participants were housewives (81.31%) and had a senior high school diploma (30.84%). Of all participants, 52.34% had children with no recent history of diarrhea, and the last time their children had diarrhea was more than one year ago (69.35%).

Figure 1 depicts the participants' level of knowledge regarding diarrhea in children. The findings revealed that the community had a good understanding of the definition of diarrhea, with 87.85% of participants correctly defining diarrhea as more frequent and loose/watery stools. This result was higher than that found in other studies done in Bandung, Indonesia (60%), Northwest Ethiopia (65.4%), and Pakistan (72%), but lower than the study conducted in Diredawa, Eastern Ethiopia (92.5%) (Mumtaz *et al*., 2014; Kosasih *et al*., 2015; Desta *et al*., 2017; Workie *et al*., 2018). This variation could be related to various sociodemographic factors and access to diarrhea information from health facilities or the media (Desta *et al*., 2017; Workie *et al*., 2018).

A study in Surabaya, Indonesia, found that the typical clinical characteristics of acute diarrhea in children were vomiting (72.67%) and fever (59.33%) (Imanadhia et al., 2019). The majority of the respondents (89.72%) also know about the symptoms following diarrhea. Abdominal pain, fever, and vomiting may also follow diarrhea caused by infections (NIDDKD, 2021).

However, there was still a lack of knowledge about how to properly provide treatment for diarrhea, particularly when it comes to determining which drug to administer; 35.51% of the participants gave loperamide to their children, with more than half of them (55.14%) were not sure about the administration. Children with acute or persistent diarrhea do not benefit from antidiarrheal medications. Some medications usually have dangerous side effects (WHO, 2005). Loperamide has been linked to severe side effects in children under three, including mortality, ileus, and lethargy. It could be an effective adjunct for oral rehydration and early refeeding in children over three with no or minimal dehydration (Li *et al*., 2017). The Food and Drug Administration in the United States has approved loperamide in children over two. The National Agency for Drug and Food Control of Indonesia, or BPOM, has approved it for children over four.

**Table 1.** Socio-demographics participants (n = 107) and its relationship with knowledge and attitudes

| Characteristics | (%) | Correlation with knowledge p-value | Correlation with attitude p-value |
|---|---|---|---|
| Gender | | | |
| Male | 10.28 | | |
| Female | 89.72 | | |
| Occupation | | 0.83 | 0.36 |
| Government employee | 0.93 | | |
| Private employee | 4.67 | | |
| Housewife | 81.31 | | |
| Enterpriser | 5.61 | | |
| Others | 7.48 | | |
| Level of Education | | 0.20 | 0.43 |
| Drop out of primary school | 17.76 | | |
| Elementary school | 16.82 | | |
| Junior high school | 27.10 | | |
| Senior high school | 30.84 | | |
| College or higher | 4.67 | | |
| Others | 2.81 | | |
| Children's History of Diarrhea | | 0.18 | 0.02[a] |
| Yes | 47.66 | | |
| No | 52.34 | | |

[a]p-value ≤ 0.05 was considered correlated

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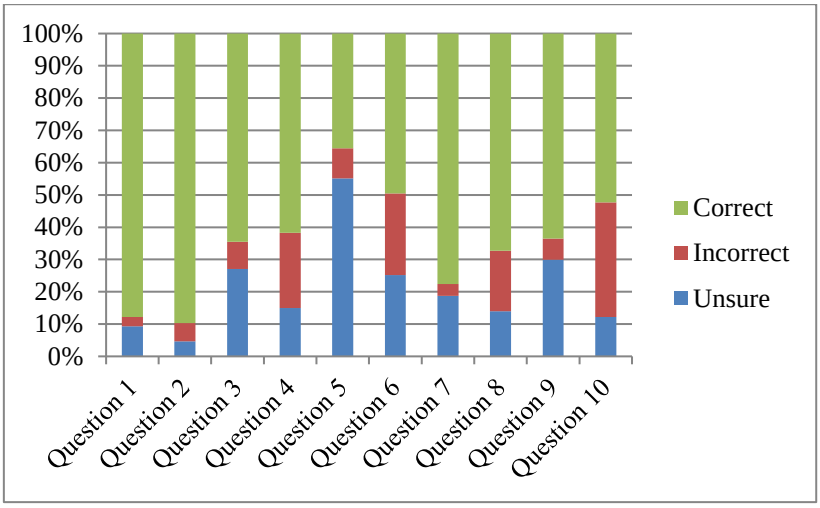
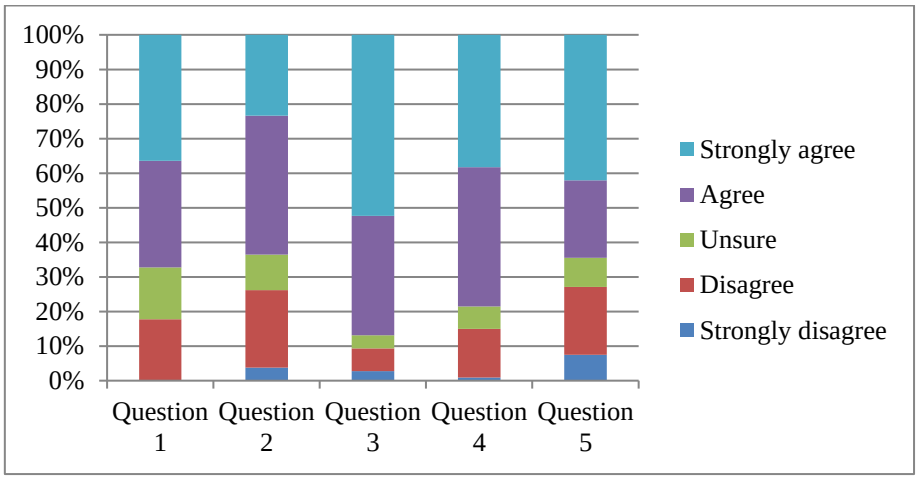



(Q1) Diarrhea is characterized by more frequent and loose/watery stools; (Q2) vomiting, fever, and abdominal pain usually following the symptoms of diarrhea; (Q3) dehydration is a condition when someone lack fluids and is characterized by less amount of urine; (Q4) oral rehydration solution was only given to children who have severe diarrhea; (Q5) loperamide can be given to children to stop diarrhea; (Q6) green vegetables and fibrous foods can help to stop diarrhea; (Q7) oral rehydration solution is a sugar solution mixed with salts; (Q8) paracetamol can be given to children who have diarrhea followed by fever; (Q9) sunken eyes, wrinkled skin, weakness, not urinating for a long time are signs of severe dehydration; (Q10) diarrhea is not a contagious disease.

**Figure 1.** Respondents' knowledge about diarrhea in children




(Q1) I can make an oral rehydration solution by myself; (Q2) I can buy an oral rehydration solution at the nearest pharmacy without a prescription; (Q3) If the diarrhea has been unresolved for more than three days, I will take my children to the physician; (Q4) I will prepare some glasses of oral rehydration solution when my child has diarrhea; (Q5) I will start taking my child to the physician when my child has sunken eyes, wrinkled skin, is weak, and has not urinated for a long time.

**Figure 2.** Respondents' attitude toward diarrhea in children

Next, more than half of the participants (61.68%) had the wrong answer. They would only provide oral rehydration salts (ORS) to their children when they have severe diarrhea. ORS provision is essential in managing children with diarrhea (WHO, 2005). UNICEF and the WHO recommended fluid replacement begin at home and be given to the childduring the diarrhea episode (UNICEF & WHO, 2009). Correct ORS preparation and provision presents sufficient water and electrolytes to correct deficits associated with acute diarrhea (WHO, 2005). The American Academy of Pediatrics recommends giving ORS to children with mild to moderate dehydration to children who have diarrhea. At the same time, children who have severe dehydration should be treated with intravenous fluids (Boluyt *et al*., 2006).

Figure 2 describes the participants' attitudes on diarrhea in children. When a child develops diarrhea, most participants had a 'positive' attitude, with 78.51% agreeing that an oral rehydration solution should be given. This result was higher than UNICEF data in 2017 stated that only 36.10% of children under five

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years old received ORS. ORS is a cost-effective treatment and reduces symptoms, severity, and diarrhea duration in children (UNICEF, 2018). Moreover, ORS could prevent up to 93% of deaths from diarrhea cases (UNICEF & WHO; 2013).

The participants also responded that if their children's diarrhea lasted more than three days, they would take them to the doctor (86.92%). This attitude is following the rule from WHO that suggests taking the child to a health worker if the child's condition does not improve in three days (WHO, 2005). Another study showed that 52.5% of mothers would bring their child to the physician after two days (Mumtaz *et al*., 2014).

However, 27.11% of participants disagreed with taking their child to the physician when their child has sunken eyes, wrinkled skin, is weak, and has not urinated for a long time. A child with severe dehydration must be urgently referred to a hospital and given ORS on the way (WHO, 2014). Untreated dehydration can be very dangerous, especially for babies, toddlers, and children, resulting in death (CPS, 2003). Dehydration in children has higher risks and is more life-threatening than in adults because a 'child's body contains a more significant proportion of water (UNICEF & WHO, 2009).

Parents should know about the signs and symptoms of diarrhea and dehydration, how to prevent dehydration at home, and the signs that indicate the children should be taken to a healthcare provider (WHO, 2005). Their knowledge influences mothers' management of diarrhea in their children. Better knowledge results in better management of diarrhea (Herwindasari, 2014). Health education can increase this knowledge through interactive discussions with health workers, adopting technology, and small group discussions (Alfira *et al*., 2019; Thobari *et al*., 2021).

## CONCLUSION

The respondents had good knowledge of recognizing the signs and symptoms of diarrhea in children. They also had a positive attitude toward diarrhea management in children. However, there was still a lack of knowledge about how to manage diarrhea properly.

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## AUTHOR CONTRIBUTIONS

Conceptualization, A.D.P.; Methodology, A.D.P., L.Y.Y; Validation, N.W.; Formal Analysis, N.W.; Investigation, N.W.; Resources, N.W.; Data Curation, B.M.P.; Writing - Original Draft, N.W., B.M.P.; Writing - Review & Editing, N.W., A.D.P.; Visualization, L.Y.Y; Supervision, A.D.P.; Funding Acquisition, A.D.P.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Effect of Sodium Alginate Concentration on Characteristics, Stability and Drug Release of Inhalation Quercetin Microspheres

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***Abstract***

***Background****: Quercetin is a flavonoid compound that has anti-inflammation activity. However, poor stability presents significant problems for the formulation into dosage forms. Microspheres are one of the potential lung delivery systems because of their ability to encapsulate various types of drugs, protect drugs from environmental effects and can release drugs in a sustained release.* ***Objective****: In the present study, the microsphere inhalation system of the anti-inflammation drug, quercetin was developed and evaluated to achieving the targeted delivery of these drugs to the lung.* ***Method****: The drug-loaded ca-alginate microspheres were prepared by aerosolization ionic gelation technique followed by freeze-drying.* ***Result****: The result of this study showed that particle size was less than 2 µm, the yield ranged from 41.33-76.14%, drug loading was less than 6%, entrapment efficiency ranged from 74.153% - 93.805% and flow properties showed that all formula had an excellent flow. Spherical microspheres were demonstrated by formulations containing 1 and 1.5% sodium alginate. A drug release study showed that the highest drug release of 30.649% was from the formulation with 2.5% sodium alginate, and the lowest drug release of 26.625% was from the formulation with 2% sodium alginate. , A stability study at temperatures of 25°C and 40°C for 28 days showed a decrease in drug loading and entrapment efficiency but an increase in particle size. The formulation containing 1.5% sodium alginate showed the optimal formula.* ***Conclusion****: These findings indicated that quercetin ca-alginate microspheres are the potential for inhalation to be delivered to the lung.*

***Keywords****: quercetin, microspheres, physical characteristic, release, stability*

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## INTRODUCTION

Acute pulmonary injury (ALI) is an inflammatory disease characterized by the overproduction of inflammatory factors in lung tissue, followed by non-cardiogenic dyspnea, severe hypoxemia, and pulmonary oedema, leading to high morbidity and mortality (Huang *et al*., 2015). ALI is also experienced by COVID-19 patients. Anti-inflammatory drugs are an alternative to avoid spikes in cytokine levels (cytokine storms) in an effort to combat COVID-19.

Quercetin is a flavonoid compound that has been shown to have anti-inflammatory effects. Research showed that quercetin increased the expression of IL-10 and Heme-oxygenase 1 by inhibiting the activation of the NLRP3 inflammasome and the secretion of inflammatory factors. These results suggest that quercetin may be a good candidate for direct lung delivery for the treatment of COVID-19 (Saeedi-Boroujeni & Mahmoudian-Sani, 2021).

The inhalation route provides many advantages over the oral route, as the high surface area with fast absorption because of increased vascularity can avoid the first pass effect, reduce dose, decrease systemic absorption and reduce side effects (Paranjpe & Müller-Goymann, 2014). However, the use of quercetin is still limited because quercetin shows low physical stability, rapid hydrolysis in aqueous solution, and is unstable because of oxygen and light (Cunico *et al*., 2020). One approach to improve the limitations in terms of the stability of quercetin is to produce microspheres.

Biodegradable polymers are often used in the manufacture of microparticles, both microspheres and microcapsules, as a support for the delivery of bioactive compounds to their targets (Soni et al., 2010). Microspheres are multiparticulate drug delivery systems designed to produce controlled drug delivery, to increase bioavailability, stability, and target drugs to specific sites. In addition, microspheres can protect drugs from environmental effects such as humidity, heat, and oxidation, and mask unpleasant tastes and odours, making them very suitable for unstable compounds such as quercetin (Uyen et al., 2020).

Sodium alginate is a natural polymer used for the manufacture of microparticles. The advantages of using sodium alginate polymer are that it is non-toxic, biodegradable, biocompatible, and relatively inexpensive (S et al., 2015). Sodium alginate can be crosslinked in an aqueous solution with divalent cations (e.g. $Ca^{2+}$, $Ba^{2+}$ and $Sr^{2+}$) to form hydrogels (Kyzioł *et al*., 2017). $Ca^{2+}$ is the most often used ion because it has non-toxic properties (Hariyadi & Hendradi, 2020a).

Characterization and study of the release of a microsphere are influenced by several factors, such as the type of polymer used, polymer concentration, polymer ratio, type of crosslinker, the concentration of crosslinker, and the method of manufacture. Athamneh *et al*. (2019) find that the morphology of sodium alginate-hyaluronic acid microspheres is spherical, and the size distribution of different microspheres is found depending on the composition of the polymer solution, its dynamic viscosity, and the stirring rate during the ionic gelation process. Hariyadi *et al*. (2019) produced microspheres ranging in size from 1.23 µm - 1.43 µm by manufacturing ciprofloxacin microspheres with alginate polymer for inhalation delivery with several polymer concentrations and aerosolization techniques. The higher the alginate concentration, the particle size also increases. An increase follows in alginate content in the diameter of the microspheres, which increases the viscosity of the alginate solution used so that large alginate droplets are formed when adding alginate solution to the crosslinking solution and causing the resulting microspheres to be more significant. In addition to particle size, Alipour *et al.* (2010) found the effect of the drug to polymer mass ratio for drug loading and entrapment efficiency of sodium alginate-paclitaxel microspheres for inhalation. Drug loading and encapsulation efficiency of microparticles depend on the manufacturing conditions. Among all the formulations made, maximum drug loading and encapsulation efficiency of up to 61% are obtained, with the highest mass ratio of paclitaxel to alginate and the highest external oil phase volume. These results are following the results of other researchers who study the effect of the mass ratio of the drugs to the polymer, the volume of the external oil phase and $CaCl_2$ to mass ratio of alginate in the production of microparticles using alginate (Alipour *et al*., 2010).

This study aimed to formulate quercetin in ca-alginate microspheres system using sodium alginate as a polymer and calcium chloride as a crosslinker. Increasing the concentration of sodium alginate was carried out to see its effect on physical characteristics, release and physical stability.

## MATERIALS AND METHODS

### Materials

Materials used in this research (Table 1) are quercetin, natrium alginate *pharmaceutical grade* (Sigma-Aldrich inc), $CaCl_2$, aquadest, ethanol 95%, and maltodextrin (Bratachem Chemistry).

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**Table 1.** The formula of microsphere quercetin-alginate

| Component | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Quercetin | 0.2% | 0.2% | 0.2% | 0.2% |
| Sodium Alginate | 1% | 1.5 % | 2% | 2.5% |
| $CaCl_2$ | 5.5% | 5.5% | 5.5% | 5.5% |
| Maltodextrin | 5% | 5% | 5% | 5% |

The drug-loaded ca-alginate microspheres were prepared by aerosolization ionic gelation technique followed by freeze-drying. Solution of sodium alginate was made separately with different concentrations of as much as 100 mL of aquadest and then stirred using a magnetic stirrer. Quercetin (0.2 gram in 20 mL ethanol) was added to the sodium alginate solution, which had been formed slowly and then stirred using a magnetic stirrer until it is homogeneous. A 5.5 % CaCl2 solution was prepared as a cross-link in 100 mL of distilled water. Quercetin alginate solution was sprayed using aerosol spray into $CaCl_2$ solution with a distance of 8 cm from the solution's surface and a pressure of 40 psi while stirring with a magnetic stirrer for two hours at a speed of 1000 rpm. The microspheres formed were separated from the $CaCl_2$ solution by centrifugation at 2500 rpm for six minutes and then washed with aquadest. The microspheres were resuspended in 5% maltodextrin solution as a lyoprotectant. The quercetin microsphere suspension was dried by freeze-dryer at -50ºC for 96 hours.

**Physical characterization of microspheres**

**Morphology**

The shape and surface of the ca-alginate quercetin microspheres were observed by scanning electron microscopy (SEM) (Hariyadi & Hendradi, 2020a).

**Particle size**

Particle size measurements were carried out using an optical microscope and the optic lab software (Hariyadi & Hendradi, 2020b).

**Yield**

Yielding close to 100% indicates that the method used in the preparation of microspheres produces the maximum number of microspheres (Kumar & Suresh, 2018). The quercetin microspheres were weighed, and the percentage yield of the prepared microspheres was calculated using the following formula:

$$yield = \frac{microsphere\ mass}{mass\ of\ polymer+drug+lyoprotectant} \text{ x } 100\%$$

The amount of quercetin trapped in the microsphere system was determined directly by calculating the total concentration in the microspheres against the theoretical content of quercetin added to the formula. The quercetin content was determined by dissolving 100 mg of quercetin-na alginate microspheres in 100 mL of phosphate buffer pH 7.4 under sonication for 60 minutes until the microspheres were completely dissolved. After that, the sample was filtered and analyzed spectrophotometrically at a wavelength of 370 nm. The experiment was replicated three times (Hazra *et al*., 2015).

$$\text{DL} = \frac{Drug\ mass\ in\ microspheres}{Microspheres\ sample\ mass}\ x\ 100\%$$

$$\text{EE} = \frac{Experimental\ drug\ mass\ in\ sample}{Hypothetical\ drug\ mass}\ x\ 100\%$$

**Flow properties**

**Bulk density dan tapped density**

Bulk density was carried out by inserting quercetin ca-alginate microsphere powder into a 100 mL graduated cylinder and weighed, then the initial volume was noted (before it was compressed). After that, the quercetin ca-alginate microsphere powder in a 100 mL graduated cylinder was compressed using a motorized tapping device, tapping 500 times and observing the final volume of the powder. Then the bulk density and tapped density were calculated using the following formula:

$$\text{bulk density} = \frac{mass\ powder\ (gram)}{initial\ volume\ (ml)}$$

$$\text{tapped density} = \frac{mass\ powder\ (gram)}{tapped\ volume\ (ml)}$$

**Carr index dan hausner ratio**

Carr's index and Hausner ratio can be determined after performing bulk density and tapped density tests, using the following formula:

$$\text{carr's index} = \frac{tapped\ density - bulk\ density}{tapped\ density} \text{x } 100\%$$

$$\text{Hausner ratio} = \frac{tapped\ density}{bulk\ density}$$

**Drug release study**

Drug release from ca-alginate microspheres was determined in phosphate buffer saline solution (pH 7.4). The release study was carried out using a thermoshaker at 37ºC at 100 rpm. A number of microspheres equivalent to 30 mg of quercetin were weighed, and the sample was put in 100 ml of phosphate buffer saline solution (pH 7.4) and then put in a thermoshaker that had reached a temperature of 37ºC and rotated at a speed of 100 rpm. Samples were taken (5 mL). Samples were taken by replacing the release medium with 5 mL of PBS solution (pH 7.4). Samples were taken after 30 minutes, 1 hour, 1 hour, 30 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, and 10 hours. At each sampling, the release medium was replaced with the same medium. Samples were filtered using 0.45 µm Millipore filter paper. The absorbance of the sample was observed using a UV-Vis

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spectrophotometer at a wavelength of 370 nm. The level of quercetin was determined by entering the absorbance value of the sample into the quercetin standard curve equation that had been made previously (Hariyadi & Hendradi, 2020a).

**Stability study**

An accelerated stability test was carried out on ca-alginate quercetin microspheres. The microsphere powder was put into a vial. These bottles were stored in a room with a temperature of 25ºC ± 2ºC and 40ºC ± 2ºC, RH 75 ± 5% for 28 days. Microsphere organoleptic changes, drug loading and powder morphology were observed to check the stability of dry powder inhalation. Quercetin ca-alginate microspheres were declared stable when organoleptically, they did not change color and did not agglomerate, the morphology of the microspheres remained spherical, the particle size remained constant and the drug loading did not decrease (Aashigari *et al*., 2019).

## RESULTS AND DISCUSSION

Based on the result of morphology and shape, formula 1 and 2 had a spherical shape and smooth surface (Figure 1). These results indicate that the two formulae are formed into a system of microspheres, this is expected to facilitate microsphere uptake by alveolar macrophage (Vishwa *et al*., 2021).

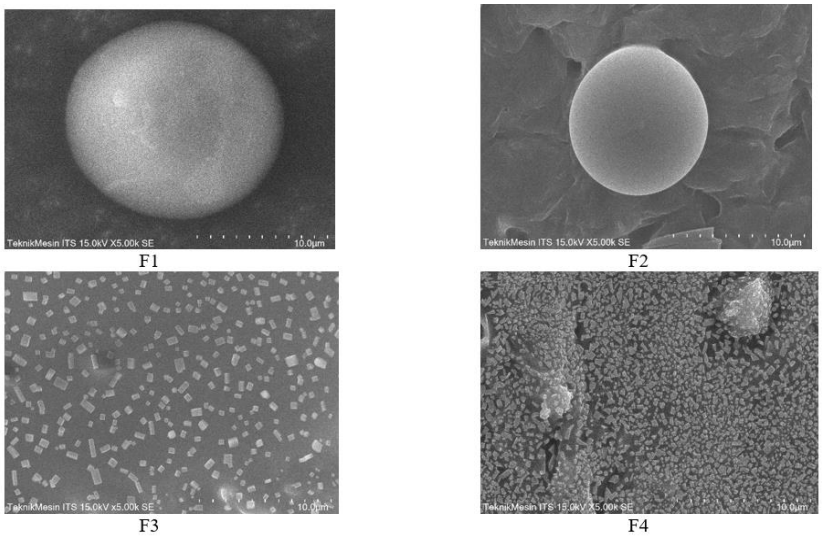


**Figure 1.** Surface morphology of a quercetin-loaded ca-alginate microsphere (Magnification 5000x)

**Table 1.** Characteristics of quercetin ca-alginate microspheres

| Formula | Particle size (µm) | Yield (%) | Drug Loading (%) | Entrapment efficiency (%) |
|---|---|---|---|---|
| 1 | 1.267 ± 0.081 | 40.80 ± 1.11 | 5.42 ± 0.105 | 74.15 ± 1.613 |
| 2 | 1.357 ± 0.092 | 57.68 ± 3.97 | 5.49 ± 0.311 | 92.95 ± 3.333 |
| 3 | 1.433 ± 0.006 | 58.83 ± 4.92 | 4.07 ± 0.074 | 92.16 ± 6.265 |
| 4 | 1.743 ± 0.120 | 54.55 ± 2.94 | 4.11 ± 0.182 | 93.81 ± 4.220 |

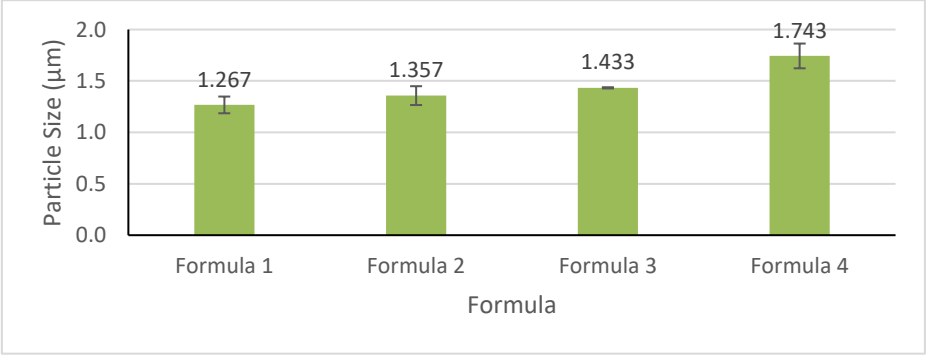


**Figure 2.** Histogram of particle size of quercetin-ca alginate microspheres with increasing polymer concentration F1 (1% sodium alginate) F2 (1.5% sodium alginate), F3 (2% sodium alginate) and F4 (2.5% sodium alginate). Data are the mean of three replications ± SD

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Particle size plays an important role in the phagocytosis of micron-sized particles in lung delivery. Based on Table 1, particles obtained for all formulas were F1 (1.1267 m), F2 (1,357 m), F3 (1,433 m) and F4 (1,743 m). The one way ANOVA statistical test for particle size distribution showed that the value of sig = 0.003 <0.05, which means that there is a significant difference between the formulae. Results indicated that the four formulae's particle size fulfills the optimal particle size for delivery to the lungs, which is < 6 µm (Vishwa *et al*., 2021). Based on the results obtained, there was an increase in particle size along with an increase in the concentration of sodium alginate. This is because when the concentration of sodium alginate increases, the viscosity increases, which causes the droplet size of the microspheres to become larger. The polydispersity index (PDI) for all formulae showed a value close to 0, which means that the particle size distribution is homogeneous.

Calculation of the yield obtained in the four formulae was F1 (40.80%), F2 (57.68%), F3 (58.83%) and F4 (54.55%) (Table 1). Based on the one-way ANOVA statistical test results, the value of sig = 0.003 < 0.05, which means that there is a significant difference. Yielding close to 100% indicated that the method used in the preparation of microspheres efficiently produces the maximum number of microspheres (Kumar & Suresh, 2018). Based on the results obtained, it can be seen that the higher the concentration of sodium alginate, the higher the yield. This is because the higher the concentration, the greater the amount of sodium alginate required, soto obtain more microspheres.

Based on Table 1, the drug loading obtained from the four formulae is F1 (5.428%), F2 (4.495%), F3 (4.075%) and F4 (4.112%). The one-way ANOVA statistical analysis showed the value of sig = 0.000 < 0.05, which means that there is a significant difference. From the results obtained, it can be seen that the higher the sodium alginate drug loading concentration, the smaller the drug loading obtained. This is because the viscosity of the resulting solution increases the concentration of sodium alginate, which causes many droplets that cannot load quercetin into the system. Meanwhile, for encapsulation efficiency, the results obtained were F1 (74.153%), F2 (92.952%), F3 (92.166%) and F4 (93.805%). Based on the results of the one-way ANOVA statistic, it showed that the value of sig = 0.004 < 0.05, which means that there is a significant difference. The highest entrapment efficiency is on F4. The increasing concentration of sodium alginate will increase the viscosity of the solution, causing the droplet size to be larger, resulting in an increase in entrapment efficiency (Hariyadi *et al*., 2019).

The flow properties test was determined by means of Carr's index and the Hausner's ratio. Carr's index results obtained from the four formulas based in Table 2 include F1 (8.703), F2 (7.963), F3 (6.427) and F4 (9.7), while the Hausner ratio obtained from the four formulae include F1 (1.092), F2 (1.082), F3 (1.069), and F4 (1,107).

The results obtained for the value of Carr's index and Hausner's ratio show that the four formulae are included in the excellent category. These results indicated that the carrier particles, such as microspheres, offer the potential to increase the flow of fine drug particles and help obtain uniform fine drug particles into the inhalation device.

The quercetin release test from the microspheres was carried out for 600 minutes using a thermoshaker. After each sampling, the media volume was replaced as much as the sampled volume to keep the media in sync. Until the 600th minute, the following was the amount of quercetin released from the microspheres: F1 (30.64%), F2 (29.35%), F3 (25.62%) and F4 (28.65%) (Table 3). Based on these results, it can be seen that the higher the concentration of sodium alginate, the lower the release. The one-way ANOVA statistical analysis shows that the value of sig = 0.040 < 0.05, which means that there is a significant difference between the formulae. An increase in sodium alginate concentration can cause an increase in viscosity so that the formed microspheres are thicker and have a denser surface so that the release of quercetin from the microspheres takes longer because the rate of diffusion of the release medium into the microspheres decreases.

The stability of quercetin ca-alginate microspheres was carried out at two different temperatures, 25ºC and 40ºC, for 28 days. Based on the results obtained for particle size, there was an increase in particle size in the four formulae at both 25ºC and 40ºC (Tables 6 and 7). However, the particle size in the four formulae is still> 6µm, which is a particle size requirement for inhalation delivery (Vishwa *et al*., 2021).

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**Table 2.** Flow properties of quercetin ca-alginate microspheres

| Formula | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Bulk Density | 0.199 ± 0.061 | 0.149 ± 0.037 | 0.217 ± 0.014 | 0.194 ± 0.054 |
| Tapped Density | 0.218 ± 0.066 | 0.162 ± 0.041 | 0.233 ± 0.018 | 0.217 ± 0.066 |
| Carr's index | 8.703 ± 2.384 | 7.963 ± 2.426 | 6.427 ± 2.414 | 9.7 ± 4.357 |
| Hauster ratio | 1.092 ± 0.030 | 1.082 ± 0.031 | 1.069 ± 0.028 | 1.107 ± 0.053 |

**Table 3.** Release study of quercetin ca-alginate microspheres

| **Sampling time (minute)** | **Cumulative quercetin release (%)** | | | |
|---|---|---|---|---|
| | **F1** | **F2** | **F3** | **F4** |
| 30 | 14.66 ± 1.584 | 22.924 ± 3.795 | 17.507 ± 3.079 | 16.984 ± 0.620 |
| 60 | 15.116 ± 2.121 | 26.748 ± 4.405 | 19.275 ± 2.702 | 20.403 ± 1.633 |
| 90 | 17.066 ± 1.615 | 24.719 ± 3.590 | 20.333 ± 3.192 | 21.088 ± 2.092 |
| 120 | 17.97 ± 1.781 | 24.711 ± 1.394 | 20.183 ± 2.647 | 22.698 ± 2.414 |
| 150 | 19.526 ± 0.910 | 24.907 ± 1.868 | 20.825 ± 1.029 | 21.615 ± 2.934 |
| 180 | 21.29 ± 0.496 | 24.214 ± 3.515 | 20.84 ± 2.030 | 22.138 ± 0.990 |
| 240 | 22.747 ± 1.307 | 25.775 ± 4.331 | 22.383 ± 3.578 | 23.011 ± 2.025 |
| 300 | 23.772 ± 1.643 | 26.137 ± 3.227 | 22.930 ± 1.881 | 24.145 ± 1.022 |
| 360 | 25.418 ±1.928 | 25.991 ± 3.662 | 23.94 ± 3.845 | 24.847 ± 0.727 |
| 420 | 26.748 ± 2.910 | 26.853 ± 3.651 | 24.496 ± 2.900 | 24.723 ± 1.689 |
| 480 | 28.712 ± 1.777 | 28.099 ± 4.727 | 26.114 ± 4.173 | 26.627 ± 2.564 |
| 540 | 29.757 ± 2.404 | 27.902 ± 4.551 | 25.371 ± 2.068 | 27.185 ± 3.190 |
| 600 | 30.649 ± 1.886 | 29.358 ± 1.654 | 25.625 ± 2.544 | 28.65 ± 3.016 |

**Table 4.** Stability study of drug loading at 25°C

| **Formula** | **Drug Loading (%)** | | | |
|---|---|---|---|---|
| | **Temperature (25°C)** | | | |
| | D0 | D7 | D15 | D28 |
| 1 | 5.428 | 5.349 | 5.432 | 5.211 |
| 2 | 4.495 | 4.795 | 4.088 | 4.157 |
| 3 | 4.075 | 3.991 | 3.963 | 4.047 |
| 4 | 4.112 | 3.991 | 3.989 | 4.116 |

**Table 5.** Stability study of drug loading at 40°C

| **Formula** | **Drug Loading (%)** | | | |
|---|---|---|---|---|
| | **Temperature (40°C)** | | | |
| | D0 | D7 | D15 | D28 |
| 1 | 5.428 | 5.030 | 4.518 | 4.421 |
| 2 | 4.495 | 4.463 | 4.158 | 4.089 |
| 3 | 4.075 | 4.061 | 4.033 | 3.964 |
| 4 | 4.112 | 4.006 | 3.992 | 3.978 |

**Table 6.** Stability study of particle size (µm) at 25°C

| **Formula** | **D0** | **D7** | **D15** | **D28** |
|---|---|---|---|---|
| 1 | 1.267 | 1.718 | 1.971 | 2.11 |
| 2 | 1.357 | 1.573 | 2.071 | 2.435 |
| 3 | 1.433 | 1.639 | 2.145 | 2.549 |
| 4 | 1.743 | 2.378 | 2.625 | 2.65 |

**Table 7.** Stability study of particle size (µm) at 40°C

| **Formula** | **D0** | **D7** | **D15** | **D28** |
|---|---|---|---|---|
| 1 | 1.267 | 1.512 | 1.768 | 1.976 |
| 2 | 1.357 | 1.784 | 1.691 | 1.92 |
| 3 | 1.433 | 1.722 | 1.779 | 1.953 |
| 4 | 1.743 | 1.745 | 1.79 | 1.955 |

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**Table 8.** Stability study of entrapment efficiency at 25°C

| Formula | Entrapment efficiency **(%)** | | | |
|---|---|---|---|---|
| | **Temperature (25°C)** | | | |
| | **D0** | **D7** | **D15** | **D28** |
| 1 | 76.277 | 75.688 | 76.864 | 73.728 |
| 2 | 91.724 | 89.906 | 76.662 | 77.960 |
| 3 | 95.674 | 93.405 | 92.757 | 94.701 |
| 4 | 96.063 | 95.401 | 94.739 | 92.380 |

**Table 9**. Stability study of entrapment efficiency at 40°C

| Formula | **Entrapment efficiency (%)** | | | |
|---|---|---|---|---|
| | **Temperature (40°C)** | | | |
| | **D0** | **D7** | **D15** | **D28** |
| 1 | 76 277 | 71.181 | 63.929 | 62.557 |
| 2 | 91.724 | 83.673 | 77.960 | 76.662 |
| 3 | 95.674 | 95.026 | 94.377 | 92.757 |
| 4 | 96.063 | 95.732 | 95.401 | 95.073 |

The stability of quercetin ca-alginate microspheres was carried out at two different temperatures, namely 25ºC and 40ºC, for 28 days. Based on the results obtained for particle size, there was an increase in particle size in the four formulae at both 25ºC and 40ºC (Tables 6 and 7). However, the particle size in the four formula**e** is still> 6 µm which is a particle size requirement for inhalation delivery (Vishwa *et al*., 2021).

Drug loading and entrapment efficiency of quercetin ca-alginate microsphere stability test at 25ºC and 40ºC decreased levels in the four formulae (Tables 4 and 5). This was because, during storage, quercetin was degraded due to the influence of temperature and storage time, so that quercetin was released from the system, namely microspheres, resulting in a decrease in drug loading and encapsulation efficiency (Tables 8 and 9).

## CONCLUSION

The quercetin-loaded ca-alginate microspheres were prepared by aerosolization ionic gelation technique followed by freeze drying. From the result, there are three things that can be concluded, that is an increase in the concentration of sodium alginate (1%, 1.5%, 2% and 2.5%) caused an increase in particle size, yield, and entrapment efficiency, while the drug loading decreased; an increase in the concentration of sodium alginate (1%, 1.5%, 2% and 2.5%) caused the release of quercetin from the microspheres to be slower; and increasing the concentration of sodium alginate (1%, 1.5%, 2% and 2.5%) in the stability test for 28 days caused a decrease in drug loading levels and entrapment efficiency, while the particle size increased.

Result showed that optimal formula containing 1.5% sodium alginate produced spherical, very good flow and small particle size fulfills dry powder inhaler characteristics with high loadings and efficiency. The optimum quercetin-ca alginate microspheres may be potential for lung inhalation delivery.

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This research is part of the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia.

## AUTHOR CONTRIBUTIONS

Conceptualization, N.R.; Methodology, D.M.H.; Validation, A.M.; Formal Analysis, T.K.; Investigation, T.K.; Resources, D.M.H.; Data Curation, T.K.; Writing - Original Draft, T.K.; Writing - Review & Editing, N.R.; Visualization, T.E.; Supervision, H.T.; Funding Acquisition, D.M.H.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Characteristic and Physical Stability of Anti-Aging Green Tea Extract (GTE) on NLC with Argan Oil as Liquid Lipid

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***Abstract***

***Background****: Green tea extract is a hydrophilic antioxidant that is difficult to penetrate. A nanostructured lipid carrier (NLC) delivers a system consisting of solid-liquid lipids that can improve penetration. Argan oil is a vegetable oil that can be used as a liquid lipid in NLC, reducing particle size and increasing penetration by hydrating the skin.* ***Objective****: To determine the formula of NLC green tea extract (NLC-GTE) with liquid lipid argan oil, which has good characteristics and is stable.* ***Methods****: Preparation of NLC-GTE used the High Shear Homogenization with solid lipids (cetyl palmitate-glyceryl stearate) - liquid lipids (argan oil) NLC-GTE1 (50:50), NLC-GTE2 (70:30), and NLC-GTE3 (90:10). Characteristic tests included organoleptic, pH, particle size (PS), and polydispersity index (PI). The physical stability test (organoleptic, pH, PS, and PI) used the thermal cycling method (3 cycles six days).* ***Result:*** *NLC-GTE1 – NLC-GTE2 has an odor of argan oil. NLC-GTE3 has odorless. NLC-GTE1 – NLC-GTE3 has a pH scale from 5.782-5.784; PS ranges from 359.73–432.56 nm; PI ranges from 0.175-0.257. The statistical analysis results showed no significant difference between NLC-GTE1 – NLC-GTE3 in pH and PI, there was a significant difference in PS NLC-GTE1; NLC-GTE2 against NLC-GTE3. Physical stability test NLC-GTE2 – NLC-GTE3 phase separation occurs. The statistical analysis results showed no significant difference in pH values NLC-GTE1 – NLC-GTE3 before and after storage; there was a significant difference in NLC-GTE3 before and after storage.* ***Conclusion:*** *NLC-GTE1 was a formula with good characteristics and stability.*

***Keywords****: argan oil, green tea extract, nanostructured lipid carrier (NLC)*

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## INTRODUCTION

Green tea extract is a plant material with antioxidant and antiaging properties that have been widely used in cosmetic products for many years (Yapar et al., 2013). Green tea extract contains four catechin derivatives with pharmacological activities: epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin-3-gallate (EGCG). Green tea catechin derivatives have been reported to have antioxidant, anti-inflammatory, and anticancer effects. The antioxidant properties of green tea extract catechins can neutralize reactive oxygen species (ROS), thereby preventing lipid peroxidation and photoaging (Gianeti et al., 2013). EGCG is an effective antioxidant is most commonly found in green tea (Avadhani et al., 2017). EGCG has been shown in vitro and in vivo to prevent oxidative damage and depletion of antioxidant enzymes caused by exposure to solar UV radiation. Topical treatments containing EGCG have reduced the skin's inflammatory response to sun exposure. It happened by inhibiting inflammatory leukocyte infiltration and production of prostaglandin metabolites, which were used to protect against sun-induced suppression of the skin immune system and prevent skin photoaging by reducing matrix expression metalloproteinases triggered by solar UV radiation (Scalia et al., 2014). EGCG has several limitations, namely easy oxidation (photodegradation) and hydrophilic properties so that penetration in the skin is low (log P 1.1) (Avadhani et al., 2017; Rosita et al., 2019).

NLCs were fabricated using solid lipids and liquid lipids, leading to specialized nanostructures with enhanced properties for therapeutic loading, altered drug release profile, and stability (Paruvathanahalli et al., 2016). One of the topical delivery systems that can be used to increase drug penetration into the skin and protect the active ingredients that are easily oxidized is nanostructured lipid carriers (Paruvathanahalli et al., 2016; Czajkowska-Kosnik., 2018). The presence of this liquid lipid can reduce the regularity of the crystal lattice. Thus, a larger space can be created to trap the active ingredients, thereby increasing the entrapment effectiveness of the NLC (Soerarti et al., 2019). The Selection of components that make up the NLC delivery system is one of the essential factors. The type of liquid lipid in the NLC composition affects nanoparticles physicochemical properties, particle size and drug distribution (Saedi et al., 2018). NLC remains solid by controlling the level of liquid lipid added in the NLC constituent formulation. A controllable drug release property for NLC can be achieved. The choice of constituent materials and the ratio between solid and liquid lipids are essential factors in the NLC formulation. A combination of cetyl palmitate-glyceryl stearate was also used o increase the regularity of solid lipids in this study.

Argan oil is a vegetable oil that is unique and rich in unsaturated fatty acids (80%), especially oleic acid (± 45%) and linoleic acid (± 37%) (Jordan et al., 2014). It is a natural oil widely applied in industrial applications such as cosmetics due to its antioxidant, skin hydration, antiaging, and skin protection properties. Argan oil is often used as a liquid lipid in NLC delivery systems. Based on the most prominent content of argan oil, namely oleic acid, argan oil as a liquid lipid in this NLC delivery system can produce good NLC characteristics (Tichota et al., 2014). It is evidenced by previous studies that the increasing concentration of oleic acid as a liquid lipid in the NLC formula could reduce the particle size (Kelidari et al., 2017). The small particle size causes an increase in the surface area of the particles in contact with the skin, thereby increasing the occlusivity to increase the penetration rate of the active ingredients into the skin. An active ingredient penetrating the skin increases its effectiveness (Czajkowska-Kosnik et al., 2018; Muller et al., 2002). Previous studies have also reported that the formulation of NLC-based hydrogel with argan oil as a liquid lipid has been successfully formulated and produces good NLC delivery system characteristics and can increase skin hydration. The success of an NLC system as a delivery system can be measured by the size of the particles that fall into the nanosize range, and the NLC must also be stable enough that there is no phase separation and creaming. The physicochemical stability of the emulsion-based carrier system can be controlled by designing the conditions and composition of the formulation to produce NLC that has excellent and stable characteristics (Rohmah dkk., 2019).

This study aimed to determine the best green tea extract NLC formula with various variations in the composition of solid lipid (cetyl palmitate-glyceryl stearate) - liquid lipid (argan oil). It is calculated based on the pH value that falls within the skin pH range, particle size and polydispersity index, and the stability of the NLC system. It can be used to develop antiaging formulas using antiaging formulas the active ingredient of green tea extract.

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**Table 1.** Composition of the NLC-GTE with variations composition of the solid lipid (cetyl palmitate-glyceryl stearate) - liquid lipid (argan oil)

| No. | Component | Function Materials | Formula (gram) | | |
|---|---|---|---|---|---|
| | | | **NLC-GTE1 (50 : 50)** | **NLC-GTE2 (70 : 30)** | **NLC-GTE3 (90 : 10)** |
| 1 | Green Tea Extract | Active Ingredient | 0.1 | 0.1 | 0.1 |
| 2 | Cetyl palmitate-glyceryl stearate | Solid Lipid | 2.5 : 2.5 : 5 | 3.5 : 3.5 : 3 | 4.5 : 4.5 : 1 |
| 3 | Argan Oil | Liquid Lipid | | | |
| 4 | Tween 20 | Surfactan | 2 | 2 | 2 |
| 5 | Lecithin | Co-Surfaktan | | 0.5 : 0.5 | |
| 6 | Synperonic F68 | | | | |
| 7 | Buffer phospate pH 5.0 | Solvent | Ad 100 | Ad 100 | Ad 100 |

*Description = total lipids **NLC-GTE1, NLC-GTE2, and NLC-GTE3** are 10%

NLC-GTE1 = Solid Lipids: Liquid Lipids (50: 50) = Cetyl palmitate: Glyceryl stearate: Argan Oil (2.5:2.5:5)

NLC-GTE2 = Solid Lipids: Liquid Lipids (70: 30) = Cetyl palmitate: Glyceryl stearate: Argan Oil (3.5:3.5:3)

NLC-GTE3 = Solid Lipid : Liquid Lipid (90 : 10) = Cetyl palmitate : Glyceryl stearate : Argan Oil (4.5:4.5:1)

## MATERIALS AND METHODS

### Materials

The ingredients used in this study were Green tea extract (Meditea, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Airlangga University), Cetyl Palmitate (BASF), Glyceryl Stearate (Medica), Argan Oil (Olvea, France), Tween 20 (Zhang Yan, Singapore), Lecithin (Solae), Synperonic F68 (Poloxamer 188) (BASF, PT. Megasetia Agung Kimia, Indonesia), Aquademineral, $NaH_2PO_4.H_2O$, and $Na_2HPO_4.2H_2O$ (SAP, Indonesia).

### Tools

The tools used in this study included SI Analytics-pH Meter Lab 855, Ultra Turrax IKA®T25 Digital High Shear Homogenizer, analytical balance (OHAUS), hotplate stirrer, Delsa Nano Submicron Particle Analyzer Beckman Coulter.

### Method

The research included manufacturing NLC green tea extract (NLC-ETH) using the High Shear Homogenization method, characterization, and stability testing. Characteristic tests were pH, particle size (PS), polydispersity index (PI), and physical stability tests. A physical stability test was performed using the thermal cycling method. It measured the pH value, particle size (PS), polydispersity index (PI) before and after storage in 3 cycles for six days.

### Preparation of green tea extract NLC delivery system (NLC-GTE)

NLC green tea extract was prepared using the modified High Shear Homogenization (HSH) method and refers to the previous research conducted by Manea et al., (2014). Composition of the NLC-GTE with variations composition of the solid lipid (cetyl palmitate-glyceryl stearate) - liquid lipid (argan oil) can be seen in Table 1. Two different phases were prepared, namely the oil and water phases. The oil phase consisted of cetyl palmitate, glyceryl stearate, and argan oil (10% total lipid). At the same time, the aqueous phase consisted of surfactant tween 20 as the surfactant synperonic F68, lecithin (1:1 in 1%), and phosphate buffer pH 5.0.

Furthermore, both were heated at the same temperature of 70 °C for 30 minutes and then in a different container, prepared green tea extract in phosphate buffer pH 5.0 30 ml and stirred at 100 rpm at 70 °C for 10 minutes. After that, the green tea extract solution was added to the oil phase while stirring at 300 rpm for 4 minutes and then continued at 500 rpm for 6 minutes. Before mixing the two steps, the aqueous phase was stirred for 2 minutes at high speed (15000 rpm) using Ultra Turrax IKA®T25 Digital High Shear Homogenizer. Then the two phases were mixed using Ultra Turax at 15,000 rpm for 7 minutes. Thus, a green tea extract NLC delivery system was obtained (Manea et al., 2014). The composition of the NLC-GTE with variations in composition of the solid lipid (cetyl palmitate-glyceryl stearate) - liquid lipid (argan oil) can be seen in Table 1.

### Physical characteristics test of the green tea extract NLC formula

Characteristic tests were conducted on the three NLC formulas of green tea extract by visual organoleptic observation. It measured the pH value with a pH meter, particle size (PS), and polydispersity index (PI) using the DelsaTM nano submicron particle size analyzer. The organoleptic examination was carried out by visual means, including color, odor, and consistency analysis.

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**Measurement the degree of acidity (pH value)**

The pH value was measured by calibrating the pH meter using a standard buffer solution of pH 7.0. After that, the electrodes are cleaned and then dried. The next step is to dilute the sample with CO2-free mineral water. Then check the pH value using the SI Analytics tool – pH Meter Lab 855.

**Particle size (PS) and polydispersity index (PI)**

Measurement of particle size (PS) and polydispersity index (PI) was carried out using a DelsaTM nano-submicron particle size analyzer. The first stage was sample dilution. A total of 50 mg of the sample was weighed on an analytical balance. Then, the aqua mineral was added to a volume of 50.0 mL. It stirred using a magnetic stirrer for 10 minutes at a speed of 500 rpm. The next step is to determine the particle size (PS) and polydispersity index (PI) by inserting the diluted sample into a glass cuvette. Then put it in the sample holder, then observe the intensity bar listed on the monitor. If it is yellow or blue, click start on the menu bar. Next, the tool measured particle size (PS) and polydispersity index (PI). After the measurements had been taken, the particle size (PS) data in nanometers (nm) and the polydispersity index appeared.

**Physical stability test of green tea extract NLC formula (NLC-GTE)**

This physical stability test used an accelerated stability test using the thermal cycling method. In this test, the formula was stored at 40 °C for 48 hours, and then kept at a temperature of 2-8 °C for 48 hours. This experiment was repeated for three cycles (6 days). Then observations and evaluations were carried out and compared with the formula given the treatment (Erawati et al., 2019).

**Statistical analysis test**

Data obtained was tested statistically on the characteristic test (pH, PS, and PI values) using one-way ANOVA to determine the significant difference between the formulas. If the result was < 0.05, it indicated a significant difference between the formulas for each test parameter. The analysis was continued with the Post Hoc Tuckey HSD test to find out which formula has a significant difference. Statistical analysis tests were also carried out on physical stability tests (pH, PS, and PI values), which were compared before and after storage for three cycles (6 days) on each test parameter using paired sample t-test. If the results were < 0.05, this indicated a significant difference between the test results before and after storage on each test parameter.

## RESULTS AND DISCUSSION

This study is distinguished based on the ratio of solid lipids (cetyl palmitate: glyceryl stearate) and liquid lipids (argan oil), such as NLC-GTE1 (50:50) with a ratio of 2.5 g: 2.5 g: 5 g (cetyl palmitate: glyceryl stearate: argan oil) in 100 grams of NLC green tea extract, NLC-GTE2 (70:30) with 3.5 g: 3.5 g: 3 g (cetyl palmitate: glyceryl stearate: argan oil) in 100 grams of NLC green tea extract, and NLC-GTE3 (90:10) with 4.5 g: 4.5 g: 1 g (cetyl palmitate: glyceryl stearate: argan oil) in 100 grams of NLC-ETH. NLC-ETH was prepared using the high shear homogenization (HSH) method. This method works by friction mechanism and breaks up particles in a mixture of lipids, surfactants, and water at a temperature of 5-10 °C above the melting point of lipids to form an emulsion.

**1. Characteristics test results**

**Results of organoleptic examination**

The results of the organoleptic examination showed that the NLC green tea extract had good physical characteristics (homogeneous). Based on the physical characteristics test, NLC-GTE1 – NLC-GTE3 is opaque white in color, NLC-GTE1 - NLC-GTE2 has a distinctive odor of argan oil, but NLC-GTE3 does not smell; this is because NLC-GTE3 has a lower concentration of argan oil, the entire formula is NLC-GTE (1-3) has a liquid consistency. The organoleptic result of NLC-GTE1, NLC-GTE2, and NLC-GTE3 can be seen in Table 2 and then result of the Characteristic test on pH value, *particle size (PS)*, and *polidispersity index (PI)* NLC-GTE1, NLC-GTE2, and NLC-GTE3 can be seen in Table 3.

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**Table 2.** Organoleptic result of NLC-GTE1, NLC-GTE2, and NLC-GTE3

| **Organoleptic** | NLC-GTE1 **(50 : 50)** | NLC-GTE2 **(70 : 30)** | NLC-GTE3 **(90 : 10)** |
|---|---|---|---|
| Color |  |  |  |
| | White opaque | White opaque | White opaque |
| Odor | Characteristic smell of argan oil | Characteristic smell of argan oil | odorless |
| Consistency | Liquid | Liquid | Liquid |

**Table 3.** Result of Characteristic test on pH value, *particle size (PS)* and *polidispersity index (PI)* NLC-GTE1, NLC-GTE2, and NLC-GTE3

| **Testing** | NLC-GTE1 **(50 : 50)** | NLC-GTE2 **(70 : 30)** | NLC-GTE3 **(90 : 10)** |
|---|---|---|---|
| pH value | 5.784 ± 0.002 | 5.784 ± 0.001 | 5.782 ± 0.003 |
| *Particle Size (nm)* | 359.73 ± 4.214 nm | 383.93 ± 8.578 nm | 432.56 nm ± 13.822 nm |
| *Polidispersity index (PI)* | 0.175 ± 0.059 | 0.213 ± 0.012 | 0.257 ± 0.013 |

**pH measurement results**

The results of measuring the pH value of the NLC delivery system of green tea extract with various comparisons of the combination of solid lipid cetyl palmitate-glyceryl stearate with argan oil as liquid lipid, namely NLC-GTE1 (50: 50); NLC-GTE2 (70:30) and NLC-GTE3 (90:10) using a pH-meter can be seen in Table 3. Based on the pH data in Table 3, all NLC formulas of green tea extract have a pH value of 5.7 and meet the skin pH specifications of 4.5 – 6.5 (Umar, 2021). A pH value is too acidic because less than 4.0 can cause skin irritation, inflammation, and even acne. At the same time, a pH that is too high more than 7.0, can cause dry and sensitive skin, so the preparations must be within that pH range so as not to cause adverse effects. Undesirable on the user's skin (Kamila, 2017). Based on the statistical analysis tests, the results obtained a significance value of > 0.05 (0.579), which indicates no significant difference in the pH value between the NLC formulas of green tea extract.

**Measurement results of particle size (PS) and polydispersity index (PI)**

The results of PS measurements of the NLC-GTE system with various comparisons of the combination of solid lipid cetyl palmitate-glyceryl stearate with argan oil as liquid lipid were NLC-GTE1 (50: 50); NLC-GTE2 (70:30), and NLC-GTE3 (90:10) using DelsaTM Nano Submicron Particle Size can be seen in Table 3. PS test results on all NLC formulas of green tea extract have PS below 500 nm. Based on the results of statistical analysis tests, a significance value of <0.05 (0.000) indicates that there is a significant difference in PS between the NLC-GTE. The analysis continued and stated that NLC-GTE1 and NLC-GTE2 had significant differences from NLC-GTE3 to determine which formulas were significantly different. It is due to differences in the concentration of liquid lipids in the NLC formula. According to Pornputtapitak (2019), the high liquid lipid content in NLC can reduce the viscosity in the NLC, resulting in small particles size caused by low surface tension. In addition, according to Apostolou et al., 2021, with increasing liquid lipid concentration, PS will decrease. It can also be attributed to the higher amount of solid lipids than liquid lipids in an NLC constituent formula which can affect the melting process and form agglomerates during NLC production. In addition, during the solid lipid compaction process in the NLC preparation, higher concentrations of solid lipids may tend to coalesce or form aggregates, which may not break apart and appear as large particles. The measurement results of the polydispersity index (PI) of all NLC systems of green tea extract are below 0.5, which means that all formulas of the NLC system of green tea extract are homogeneous.

The above statement is also reinforced by the results of research by Ebtavanny et al (2018), that study also showed that increasing the concentration of liquid lipids in NLC could reduce PS and PI, so that the presence of argan oil as liquid lipid in NLC-GTE showed a significant role in PS and PI. Although, based on the results of the statistical analysis of PI, a significance value of > 0.05

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(0.081) was obtained, which indicated no significant difference in the polydispersity index between the NLC formulas of green tea extract. When compared between the results of research conducted by Tichota et al (2014) with the results of this study, it shows that the benefits of argan oil as a liquid lipid in the NLC delivery system can produce good characteristics in PS and PI parameters.

The presence of argan oil as a liquid lipid in NLC-GTE is considered to be able for reduce PS and PI, so those small and homogeneous particles are formed in an NLC delivery system, although compared to the results of research conducted by Manea et al., (2014) the results of PS are smaller, this may be due to the liquid lipid used in this study is Grape Seed Oil and the use of other additives that are different from this study. However, the PS and PI NLC-GTE observations with argan oil as a liquid lipid still yielded values that were in accordance with the NLC standard so that they were still declared to meet the NLC requirements with small and homogeneous particle sizes.

**2. Physical stability test results**

In this study, the physical stability test was carried outThe physical stability test was carried out in this study using the thermal cycling method. In this test, the formula was stored at 40 °C for 48 hours, and then kept at a temperature of 2-8 °C for 48 hours. This experiment was repeated for three cycles (6 days). Aspects in of the stability test were organoleptic (presence or absence of phase separation), pH value, particle size, and polydispersity index before and after storage. Result The result of Physical Stability NLC-GTE can be seen in Table 4.

The visual inspection results on all NLC formulas of green tea extract showed that there was a phase separation in F3, but this could be overcome by stirring because during three storage cycles, there was no solid phase separation, or it could not return to its original state. NLC-GTE3 has the largest particle size (432.56 nm ± 13.822), so the phase separation tends to occur faster.

The results of measuring the pH value of all NLC formulas of green tea extract during storage for three cycles (6 days) still had a pH value in the skin pH range of 4.5-6.5 (Umar, 2021). Based on the results of statistical analysis tests on the measurement of pH values, a significance value of > 0.05 showed that there was no significant between before and after storage in NLC-GTE1, NLC-GTE2, and NLC-GTE3 (Significance value NLC-GTE1 = 0.147; NLC-GTE2 = 0.300; NLC-GTE3 = 0.060) so that all NLC formulas of green tea extract were considered to have a stable pH value. The results of the physical stability test also showed that the increasing concentration of liquid lipid (argan oil) in a system did not affect the pH of the NLC green tea extract. Histogram A histogram of the physical stability test results observed pH values NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days) can be seen in the Figure 1. The content of surfactant, co-surfactant and aqueous phase (Phosphate buffer pH 5.0) in all NLC formulas of green tea extract was also in the same amount, so that this did not affect the pH value of the NLC formula of green tea extract.

The particle size results in the three NLC formulas of green tea extract storage for three cycles (6 days) still had PS below 500 nm. It meant that the NLC green tea extract was still considered to meet the PS NLC specifications that had been set and the PI results in the three formulas still had values below 0.5 which means that the NLC of green tea extract is still considered homogeneous. Based on the statistical analysis test on PS, there was no significant difference between before and after storage in NLC-GTE1 and NLC-GTE2. Yet, there was a significant difference between before and after storage in NLC-GTE3 (Significance value NLC-GTE1 = 0.318; NLC-GTE2 = 0.066; NLC-GTE3 = 0.034). Based on the statistical analysis test on PI, there was no significant difference between before and after storage in NLC-GTE1 and NLC-GTE2. However, there was a significant difference between before and after storage in NLC-GTE3 (Significance value NLC-GTE1 = 0.150; NLC-GTE2 = 0.326; NLC-GTE3 = 0.013).

A histogram of the physical stability test results observed for particle sizes NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days), can be seen in Figure 2. A histogram of the physical stability test results observing the polydispersity index NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days) can be seen in the Figure 3.

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**Table 4.** Result of Physical Stability NLC-GTE

| Time | Formula | Organoleptic | pH value | *Particle Size (nm)* | *Polidispersity Index (PI)* |
|---|---|---|---|---|---|
| Before storage | NLC-GTE1 |  | 5.705 ± 0.005 | 363.033 ± 4.404 | 0.267 ± 0.026 |
| | NLC-GTE2 |  | 5.643 ± 0.005 | 383.933 ± 8.578 | 0.268 ± 0.009 |
| | NLC-GTE3 |  | 5.797 ± 0.006 | 443.233 ± 4.966 | 0.257 ± 0.015 |
| After storage (3 cycles 6 days) | NLC-GTE1 |  | 5.713 ± 0.006 | 351.933 ± 14.189 | 0.301 ± 0.004 |
| | NLC-GTE2 |  | 5.686 ± 0.012 | 397.8 ± 4.214 | 0.280 ± 0.014 |
| | NLC-GTE3 |  | 5.754 ± 0.013 | 465.166 ± 5.396 | 0.298 ± 0.007 |

The combination of solid lipid (cetyl palmitate: glyceryl stearate): liquid lipid (argan oil) (90:10) in NLC-GTE3 was considered unable to form a stable green tea extract NLC delivery system for particle size and polydispersity index parameters. It is due to the concentration of argan oil as a liquid lipid at NLC-GTE3 (90: 10), at least compared to NLC-GTE1 and NLC-GTE2. The two things above are that the particle size and polydispersity index are closely related. It is confirmed by Suprobo dan Rahmi (2015), who state that the smaller the particle size, the more homogeneous the resulting system. The homogeneity of a system is closely related to the discovery of the system. The smaller the size of the dispersed particles, the configuration of the dispersed phase in the dispersing medium will also be regular. The higher liquid lipid content of NLC-GTE also occurs in the irregular matrix. The addition of liquid lipids also increases, which causes the crystallization process to be inhibited, so that drug release during storage can be minimized. The lower the recrystallization index of a material, the smaller the crystal regularity of the NLC system. The irregularity of the crystal lattice space can accommodate more prominent drugs so that the entrapment efficiency will be greater (Erawati et al., 2019). The presence of the liquid lipid can reduce the regularity of crystal lattice so that an NLC system has a larger space to trap the active ingredients by increasing entrapment efficiency and increasing the stability of NLC (Soerarti et al., 2019).

The statistical analysis of physical stability tests on PI NLC-GTE3 showed a significant difference between PI before and after storage, so it was concluded that NLC-GTE3 was not stable to the PI parameter. However, it can be seen from the PI value before and after storage that it was still within the specified PI value specification range. It was caused by the concentration of the combination of solid lipid (cetyl palmitate-glyceryl stearate): liquid lipid (argan oil) 90:10. It meant the solid lipid content was more than the liquid lipid in the NLC-GTE3. Thus, the high solid lipids can cause a decrease in the trapping space of the active ingredient in the system, so some active ingredients may be pushed out of the NLC system and can increase the particle size and polydispersity index. The addition of liquid lipids also increased stability caused the crystallization process to be inhibited, so drug expulsion during storage can be minimized (Erawati et al., 2019). The concentration of the combination of solid lipid (cetyl palmitate-glyceryl

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stearate) with liquid lipid (argan oil), which is 90: 10, is considered less able to form a good NLC system is not stable during storage against PS and PI.

Based on all the results of the physical stability test, it shows the stability of the NLC-GTE1 > NLC-GTE2 > NLC-GTE3 system, there is no phase separation, including the skin pH, particle size (PS), and polydispersity index (PI) according to the specifications of a homogeneous NLC system.

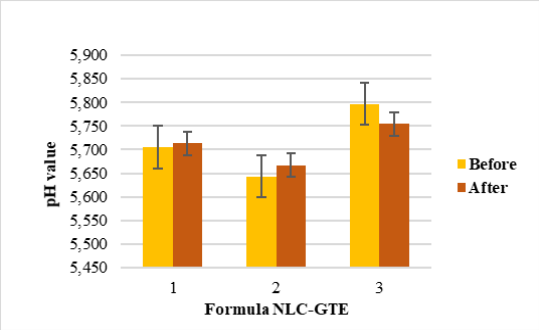


**Figure 1.** Histogram of the physical stability test results observed pH values NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days)

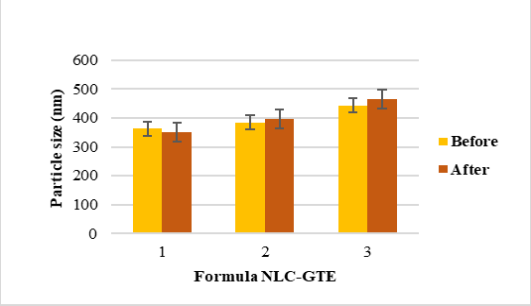


**Figure 2.** Histogram of the physical stability test results observed for particle sizes NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days)

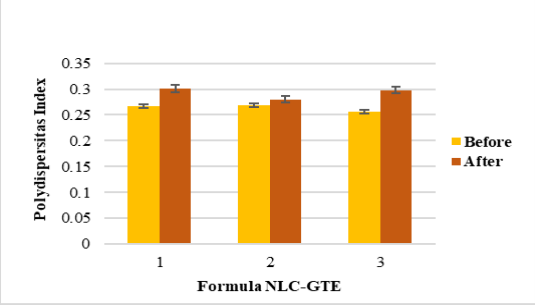


**Figure 3.** Histogram of the results of the physical stability test observing the polydispersity index NLC-GTE1, NLC-GTE2, and NLC-GTE3 before and after storage (three cycles 6 days)

## CONCLUSION

Based on the results, the NLC system characteristics showed Green tea extract with a ratio of solid lipid cetyl palmitate-glyceryl stearate and liquid lipid argan oil 50:50; 70:30 and 90:10. It includes organoleptic, pH value, particle size (PS), and polydispersity index (PI). Besides that, increasing the amount of liquid lipid argan oil in the NLC system of green tea extract can reduce PS. Green tea extract NLC formulas have pH values within the skin pH range and have a polydispersity index value below 0.5. It indicates the homogeneity of the NLC system. Based on the results, it is concluded that NLC-GTE1 has a smaller particle size than NLC-GTE2. Based on the results of physical stability, NLC-GTE1 and NLC-GTE2 are more stable than NLC-GTE3, but NLC-GTE1 is more stable than NLC-GTE2 because there is no phase separation. NLC green tea extract with a combination of solid lipid cetyl palmitate-glyceryl stearate and liquid lipid argan oil (50:50) is a formula with good and stable characteristics.

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## AUTHOR CONTRIBUTIONS

Conceptualization, A.N.S., W.S., T.P.; Methodology, A.N.S., W.S., T.P.; Validation, A.N.S.; Formal Analysis, A.N.S.; Investigation, A.N.S.; Resources, A.N.S., D.A.P.; Data Curation, A.N.S., W.S.; Writing - Original Draft, A.N.S.; Writing - Review & Editing, A.N.S., W.S., T.P.; Visualization, A.N.S.; Supervision, A.N.S., W.S., T.P.; Project Administration, A.N.S., W.S., T.P.; Funding Acquisition, A.N.S.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Anti-Hepatitis C Activity of Combination of *Ruta angustifolia* Extract and Ribavirin

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***Abstract***

***Background****: Hepatitis c virus infection is a global health problem which chronically infected 71 million people in the world. This infection has a risk of becoming liver cirrhosis and hepatocellular carcinoma. Since the current HCV therapy has been developed by direct-acting antivirals (DAA), however, most patients get limited access due to the high cost. Therefore, further development anti-HCV agent still greatly needed. Ruta angustifolia is a natural resource which was reported to possess anti-HCV activity. Ribavirin is an antiviral agent used to treat several virus infections, either DNA or RNA. Ribavirin was known to inhibit HCV infection by regulated immune system in host cells and interfering the replication of HCV by inhibit HCV RdRp.* ***Objective****: The current study evaluated the combination treatment of R. angustifolia extracts and ribavirin by in vitro culture cells of Huh 7it.* ***Method****: The study was conducted under an invitro cell culture of Huh 7it and infected with JFH1a. Result: The result demonstrated an enhancement effect of the extract by increasing the anti-HCV activity 3.5-fold higher compared to ribavirin alone. The 50% inhibitory concentration of ribavirin by single treatment was 10.43 ± 0.18 µg/mL, while in combination with Ruta angustifolia extract was 2.80 ± 0.03 µg/mL. Further analysis of the combination by CompuSyn software mediated a synergistic effect among the combination with a combination index value of 0.691.* ***Conclusion****: These results suggested that combination of Ribavirin and Ruta angustifolia should be considered in developing anti-hepatitis C virus agents.*

***Keywords****: hepatitis c virus, Ruta angustifolia, ribavirin, medicine, infectious disease*

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## INTRODUCTION

The medicinal plant is potential source for various bioactivities due to its metabolite properties, including their activities as antiviral and hepatitis (Ashfaq & Idrees, 2014; Dhama et al., 2018; Wahyuni et al., 2013a; Wang *et al*., 2018). The plant extract of *Ruta angustifolia L.* has been reported in a previous study to possess antiviral activity against hepatitis C virus with no toxic effect (Wahyuni et al., 2014). Mode of Action assay was demonstrated to inhibit dominantly in the post-entry step of HCV life cycle, and decreased the HCV NS3 level (Wahyuni et al., 2019). The isolated compounds of *R. angustifolia*, chalepin and pseudane IX exhibited to decrease viral RNA replication and reduced viral protein synthesis (Wahyuni et al., 2014). Those results demonstrated the potential of the ethanol extract of *R. angustifolia* L on HCV infection, making the extract a prospect as an anti-HCV agent. Medicinal plants are commonly used as complementary medicine to support the effectiveness of standard drugs. A combination of drugs may improve the effectiveness and decrease the resistance potency (Ulrich-Merzenich, 2014; Vickers & Zollman, 1999). A combination assay of ethanol extract of *R. angustifolia* have been done with Direct Acting Antiviral Agents (DAAs), simeprevir and telaprevir, which revealed a synergistic effect (Wahyuni et al., 2019). The combination aims to produce a synergistic or additive effect, where the impact of the combination of extracts and drugs will be greater for inhibition of the hepatitis C virus than the effect of drugs used individually (Connell et al., 2013). Conceptually, combination therapy of several hepatitis C antiviral agents with different mechanisms of action can increase antiviral effectiveness and avoid viral resistance (Chatterji et al., 2014). Ribavirin is an antiviral drug that acts on host cells (host targeting agents) by inhibiting ribonucleoprotein synthesis and interfering with the early stages of viral transcription (Rumi, 2009). Therefore, it is necessary to evaluate the combination therapy of ribavirin for anti-hepatitis C virus activity with natural ingredients, namely *Ruta angustifolia* to treat hepatitis C virus infection safely, have affordable treatment costs, have no toxicity and reduce drug resistance.

## MATERIALS AND METHODS

### Materials

The leaves of *R. angustifolia* was obtained from Jombang, East Java Indonesia and has been verified by a expert botanist researchers from Materia Medika Indonesia, Batu, East Java. Huh7it-1 hepatocyte cells and JHF1 hepatitis C virus (Cultivaed in Institute Tropical Disease, Airlangga University) were propagated as described previously (Wahyuni et al., 2013b; Wahyuni et al., 2014),). Dimethyl Sulfoxide (DMSO), medium of Dubelco's Modified Eagle Medium (DMEM, GIBCO-Invitrogen), 10% Fetal Bovin Serum (FBS, GIBCO-Invitrogen), Non-Essential Amino Acid (NEAA, GIBCO-Invitrogen), Dubelco's Phosphate Buffered Saline (DPBS, GIBCO-Invitrogen), Penicillin Streptomycin (GIBCO-Invitrogen), Trypsin-EDTA (GIBI BCO-Invitrogen), Bovine Serum Albumin (BSA, Roche), formaldehyde (HCHO, Applicant), TritonX-100 (Promega), Thermo staining 3,3'-diaminobenzidine (DAB), hepatitis patient antiserum C, HRP-Goat-anti-human *Ig* (MBL) were used for anti HCV assay. The material used in the toxicity test was MTT (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymetho xyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) reagents (Thermo fisher).

### Method

#### Collection and extraction

*R. angustafolia* was collected from Jombang area, East Java, Indonesia. The leaves of the plant were dried and extracted with 96% ethanol three times using the maceration method. The filtrate was collected and concentrated with a rotary evaporator. The extract was stored at -20°C until it used.

#### Cell and virus preparation

The cells of hepatocyte (Huh7it) were cultivated in Dulbecco's modified eagle's medium (Wako Chemicals) and supplemented with fetal bovine serum (Biowest, Inc) and non-essential amino acids/NAA (Invitrogen). The mixture of 100 IU/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cells were incubated at the condition of 37°C in a 5% $CO_2$ incubator. While the virus of hepatitis C was propagated for 3, 4 and 7 days and following by titer assay for each day (Wahyuni et al., 2014).

#### Sample preparation for anti-HCV activity

Sample preparation was started by making a stock solution of extract in dimethylsulfoxide (DMSO) to obtain a concentration of 100 mg/mL. The ribavirin was provided in a sterile water solution with a concentration of 10.000 µg/mL. The stock solutions were kept at -20°C until used.

#### Anti-HCV activity assay

The initial treatment in conducting the sample activity test was seeding cells in 48 well plates with a cell density of $5.4x10^4$ and incubate for 24 hours, JFH1 virus was added with a multiplicity of infection (m.o.i) of 0.1. The ethanol extract of leaves of *R. angustifolia*

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with a concentration of 100, 30, 10, 1, 0.1, and 0.01 µg/mL were inoculated into the cells. Ribavirin was also evaluated for its activities at the concentration of 50, 30, 10, 1, 0.1, and 0.01 µg/mL. The test was carried out to determine the $IC_{50}$ concentration of the ethanol extract of *R. angustifolia*. and ribavirin to be used as concentrations in the combination assay. Combination of *R. angustifolia* and ribavirin were determined at the concentration of 4 x $IC_{50}$; 2 x $IC_{50}$; 1 x $IC_{50}$; 1/2x $IC_{50}$; and 1/4x $IC_{50}$ of both substances. The tested-concentration of ribavirin was 2.5, 5, 10, 20, and 40 µg/mL, while the tested-concentration of *R. agustifolia* extract was 0.75, 1.5, 3, 6, and 12 µg/mL. The evaluation was carried out either ribavirin alone or in combination with extract of *R. angustifolia*. The combination method is commonly referred to the Chou-Talalay method (Chou, 2010). In each well, 100 µL of the test material was inoculated with the mixture of virus and incubated for 2 hours, then cells were rinsed, and 200 µL of test material were added and incubated for approximately 46 hours. The supernatant containing hepatitis C virus was collected and further analyzed its virus titration. The virus titration was conducted in the Huh7it cells that had been seeded in 96 well plates with a density of $2.4x10^4$ for 24 hours. After inoculation of the virus supernatant for 2 days, cells were fixed with 3.7% formaldehyde 200 µL and cell permeabilization using 0.5% triton X-100 100 µL. Observation of infected cells was carried out using DAB thermo staining: substrate (1:9). Colonies of infected cells were evaluated under the microscope (Wahyuni et al., 2018; Wahyuni et al., 2013a).

**Cytotoxicity test**

A toxicity test was performed using the reagent of MTT reagent. Huh7it cells were exposed to the test materials, the ethanol extract of the leaves of *R. angustifolia L.*, ribavirin, and a combination of the extract and ribavirin. In this toxicity test, 60 µL of each extract concentration, ribavirin and the mixture of extract and ribavirin were inoculated into the cells with the concentration of 4 x $IC_{50}$; 2 x $IC_{50}$; 1 x IC50; ½ x$IC_{50}$; and ¼ x $IC_{50}$ and incubated for 48 hours.

Then, the remaining medium was discarded, and 150 µL of medium containing MTT reagent was added and incubated for 4 hours. To dissolve the precipitate formed from the MTT reaction, DMSO was added. The absorbance was detected at 560 nm and 750 nm wavelength of the Microplate Multidetector Reader (Sigma). The absorbance of the sample was measured by comparing to the control (Wahyuni et al., 2019; Wahyuni et al., 2018).

**Data analysis**

Data of the number of infected cells with the virus will be calculated using the formula below and will get the percentage of infected cells.

$$\% \text{ Infected cells} = \left(\frac{Number\ of\ infected\ cells\ in\ sample}{number\ of\ infected\ cells\ in\ control}\right) x\ 100\%$$

To calculate % inhibition = 100% - % infected cells. The $IC_{50}$ value was further determined using a probit log analysis with SPSS. While, the data in the form of sample absorbance in the toxicity test will be calculated using the formula below and obtained % cell viability data.

$$\% \text{ Cell viability} = \left(\frac{Sample\ absorbance}{Control\ absorbance}\right) x\ 100\%$$

Furthermore, to evaluate the synergistic or additive effect of the combination of the ethanol extract of the leaves of *R. angustifolia* and ribavirin, CompuSyn software was used, and further justified based on the combination index value (<1: synergistic, 1: additive, >1: antagonist). The data needed to be included in the CompuSyn software are the concentration and % inhibition of each extract and drug as well as the concentration and % inhibition of the combination (Chou, 2010).

## RESULTS AND DISCUSSION

*R. angustifolia* has been analyzed its potency in anti-HCV activity in the previous work (Wahyuni et al., 2020; Wahyuni et al., 2019; Wahyuni et al., 2014). Further exploration its activities in the combination with Ribavirin was done in this study.

Three parallel assays of anti-HCV activities were carried out in vitro using Huh7it cells and JFH1a virus. Anti-hepatitis C activity test of the combination between the ethanol extract of the leaves of *R. angustifolia* and ribavirin on Huh7it cells and JFH1a virus was followed by the Chou-Talalay method. The results of the ed activity test of the ethanol extract of the leaves of R. angustifolia L. and ribavirin are shown in Figure 1. The combination can increase the activity, which is shown by the reduction of the $IC_{50}$ value of ribavirin from 10.43 ± 0.18 µg/mL (single treatment) to 2.80 ± 0.03 µg/mL (in combination treatment). Thus, the combination of *R. angustifolia* extract with ribavirin could increase the inhibitory activity of ribavirin against the hepatitis C virus by 3.7 times higher compared to ribavirin alone. The combination index values was obtained through the CompuSyn software analysis. The combination index (CI) values were revealed at the concentration with 50, 75, 90 and 95 % inhibition activities. All of the CI values demonstrated less than 1, which indicates that the

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combination of *R. angustifolia* extract and ribavirin produces a synergistic effect, as shown in Table 1.

Ribavirin is one of the initial HCV infection treatments with an interferon combination. It modulates T helper-1 and -2 lymphocyte imbalance, interferes with cell guanosine triphosphate by inhibiting inosine monophosphate dehydrogenase, and the viral RNA-dependent RNA polymerase, impairment of translation by preventing the capping of messenger RNA and caused viral mutagenesis. Although the current treatments use Direct Acting Antiviral agents (DAAs), however ribavirin is still use in some areas (Mathur et al., 2018). While ethanol extract of *R. angustifolia*, and its isolated compounds, chalepin and pseudane IX, were shown to decrease NS3 protease which involved in the replication of virus. Moreover, it was reported that the combination of extract of *R. angustifolia* and NS3 inhibitor demonstrated a synergistic effect (Wahyuni et al., 2019; Wahyuni et al., 2014). Therefore, the combination of ribavirin and *R. angustifolia* extract may provide a different side of actions to inhibit HCV virus. The results of the toxicity test of the combination of the ethanol extract of the leaves of R. angustifolia and ribavirin showed that the concentration-tested was not toxic to hepatocyte cells. The percentage of cells viability at all concentration-tested was higher than 90%, as shown in the Figure 2.

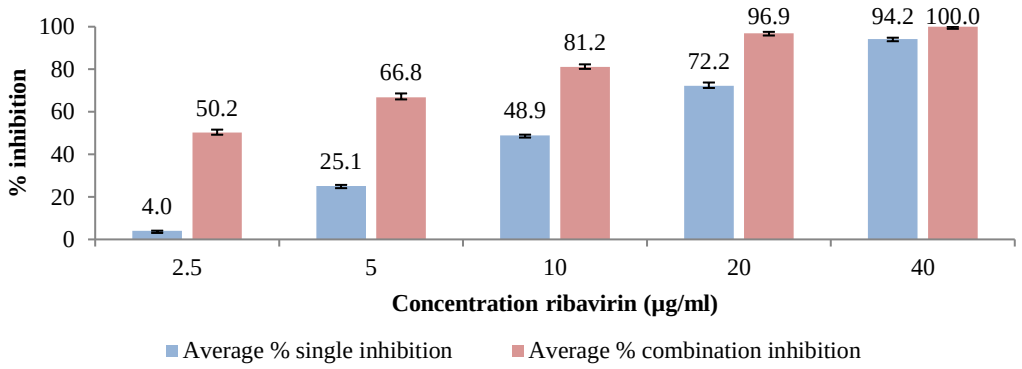


**Figure 1.** The percentage inhibition of single ribavirin and combination of ribavirin and ethanol extract of *R. angustifolia* according to the Chou-Talalay method. Data were represented 3 independent experiments

**Table 1.** The values of the combination index (CI) of the combination between the ethanol extract of the leaves of *R. angustifolia L.* and ribavirin

| Combination | Combination index (CI) value | | | |
|---|---|---|---|---|
| | $IC_{50}$ | $IC_{75}$ | $IC_{90}$ | $IC_{95}$ |
| *R. angustifolia L.* Extract + Ribavirin | 0.691 | 0.568 | 0.467 | 0.408 |

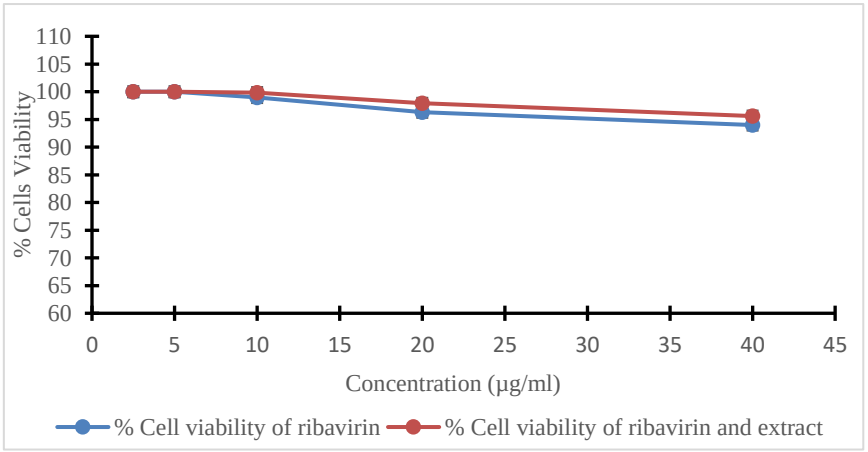


**Figure 2.** The percentage cells viability of single ribavirin and ribavirin combination with the ethanol extract of the leaves of *R. angustifolia L.* according to the Chou-Talalay method. Data were represented 3 independent experiments

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## CONCLUSION

The combination of the ethanol extract of the leaves of *R. angustifolia* with ribavirin revealed a synergistic effect which increased the anti-HCV activity 3.7 times higher compare to the ribavirin alone without any toxic effect. These results suggested that a combination of ribavirin and *R. angustifolia* should be considered in developing anti-hepatitis C virus agents.

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## AUTHOR CONTRIBUTIONS

Conceptualization, T.S.W, C.A.U., A.F.; Software, T.S.W., A.A.P.; Methodology, T.S.W., A.A.P.; Validation, T.S.W., A.W.; Writing - Original Draft, T.S.W., A.A.P.; Writing - Review & Editing, C.A.U., A.W.; Funding Acquisition, T.S.W.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Delivering a Birth Safely – Case Reports of Perineal Infection Prevention among Pregnant Women Living Around Ex-landfills

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***Abstract***

***Background****: Pregnant women living around ex-landfills have a higher risk of infection due to slum milieu, poor nutritional status, and fetal head pressure on the perineum during labor which can cause it to tear.* ***Objective****: This research aimed to emphasize the importance of limiting prophylaxis antibiotics for the 1st and 2nd degrees of perineal tears due to their negligible risk of infection.* ***Case presentations:*** *This is a report of two primigravid women aged 19 years old (cases 1 and 2) and multiparous women aged 29 and 30 yo (cases 3 and 4) who managed to give birth safely in Puskesmas (Indonesian primary healthcare facilities), despite having low blood pressure and non-adherence to antenatal care. All of them suffered from 2nd degree of perineal tears and received analgesics, iron, vitamin B complex, and vitamin A tablets. The subjects attended puerperium care on days, 14, and 42 postpartum at Puskesmas.* ***Discussion****: In March 2020, Puskesmas' healthcare team performed a new 1st and 2nd-degree perineal tears prevention without antibiotics following normal vaginal birth. Standard care consists of personal hygiene, perineal wound care, and education on the importance of nutritious food and adherence to maternal supplementation. All subjects presented with complete perineal wound healing without infection at day-42 postpartum.* ***Conclusion****: It can be concluded that 2nd-degree perineal tears infection following normal vaginal birth could be prevented without antibiotics. It is strongly suggested to control the maternal nutritional states, potentially interfering with the maternal ability to heal the perineal wound.*

***Keywords****: perineal wound, infection risk, prevention, antibiotics, covid-19*

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## INTRODUCTION

This report describes the cases of pregnant women with low social, and economic class and education living around the ex-landfills People who live near former landfills typically make a living as collectors of abandoned things, such as used bottles and cardboard stacked around their homes. This pile of used goods causes the house's surroundings to become a slum and is a source of infection transmission, which could endanger pregnant women who have a higher risk of infection due to their immunocompromised condition.

At term, the uterine contractility would become more frequent with a narrower time interval along the peripartum period causing escalating pain in the mother. During normal vaginal delivery, the perineum, vaginal, and anal tissue were possibly torn, increasing the risk of infection. The female perineum is the diamond-shaped inferior outlet of the pelvis, bordered by the pubic symphysis anteriorly and the coccyx posteriorly (Goh *et al.*, 2018). Perineal tears are classified into 4 degrees, ranging from the 1st degree of laceration of the vaginal mucosa or perineal skin only to the 4th degree of obstetric anal sphincter injury, also known as OASI. (RCOG, 2015; WHO, 2015).

The first degree of perineal tear is considered minor and could be healed without treatment, while the 2nd-degree one that requires suturing has a higher risk of infection. These two kinds of perineal tears can be treated at Puskesmas, while the major 3rd and 4th degree must be referred to the hospital due to the need for wound repair in an operating theatre. Thus the 3rd and 4th degrees of perineal tears surgery required prophylaxis antibiotics to prevent surgical wound infection (RCOG, 2015; WHO, 2015).

. The former local policy for preventing infection of perineal tears that require suturing at Puskesmas was to administer amoxicillin 500 mg three times a day and mefenamic acid 500 mg three times a day for three days postpartum. Thus, there are increasing concerns about the risk of resistance due to antibiotics overuse to prevent minor infection of 1st and 2nd degree of perineal tears following normal vaginal delivery. This research aimed to emphasize the importance of limiting the use of prophylaxis antibiotics for the 1st and 2nd degrees of perineal tears due to their negligible risk of infection.

## MATERIALS AND METHODS

This is a case report about implementing infection control without antibiotics in four pregnant women with 2nd degree of perineal tears, which require to be sutured after normal vaginal delivery at Puskesmas Keputih. This Community Health Center is located near Surabaya's ex-landfills surrounded by middle-low income and low-educated population.

In March 2020, there was a round table discussion about 1st and 2nd perineal infection prevention strategies without antibiotics use which was assisted by obstetricians from tertiary care hospital and attended by the head of Puskesmas, pharmacist, and midwives. This research collaborated with the maternal healthcare team of Puskesmas to implement the new prevention strategies. This research protocol complied with all relevant national regulations and institutional policies and is following the tenets of the Helsinki Declaration (as revised in 2013) and has been approved by the Ethics Committee of the University of Surabaya. This research holds informed consent from all the patients.

The pregnant women who delivered their babies through normal vaginal birth between March – April 2021 and had received sutured on the 1st- or 2nd-degree of perineal tears and standard care for preventing perineal wound infection were included in the study of this research. Pregnant women prescribed prophylaxis antibiotics were excluded. After informed consent was given, we measured the self-filled validated questionnaire on maternal knowledge about perineal wound care and infection prevention, prophylaxis analgesic and antibiotics used for the particular case of perineal tears, and drug adherence upon recruitment. ' 'patient's knowledge is considered as "low", "medium", and "high level of knowledge" if there was consecutive≥ 75%, >75 - 90%, and >90% correct answer to the questionnaire. Before and after standard care, the questionnaire was administered to identify maternal education needs to prevent perineal wound infection without antibiotics and administer maternal supplementation.

Then the healthcare team serves all pregnant women with new standard care without antibiotics for perineal tears infection prevention, which is consisted of education and training about:

1. personal hygiene,
2. adequate nutrition during pregnancy and 42 days postpartum (puerperium period),
3. perineal wound care,
4. early initiation of exclusive breastfeeding during the first six months of the baby's life.

Ten tablets of mefenamic acid for prophylaxis pain of perineal tears plus maternal supplementation during puerperium care consist of 2 tablets of vitamin A, 20

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tablets of vitamin B complex, and iron tablets given to the mother tablets given vaginal delivery. Pharmacists carried out education about medication administration and their important indication related to mothers' health during the puerperium period. Mother adherence to analgesic dosing regimes and maternal supplementation was observed during the puerperium period. Due to the Covid-19 pandemic societal constraints to comply with Covid-19 health standards, the same questioning technique could not be used during the postpartum visit to the Puskesmas. Since then, information has been acquired through WhatsApp conversations and phone calls. During the Covid-19 epidemic, postpartum checkups were changed from three-day intervals to seven-, fourteen-, and forty-two-day intervals.

**CASE PRESENTATIONS**

Case 1.

A 19 years-old primiparous woman attended the routine visit to the maternal clinic at Puskesmas on February 10, 2020. The 'patient's weight was 68.5 kg and proportional to the 35-36 weeks of gestational period, while the blood pressure was 100/60 mmHg. The past medical history was nausea, vomiting, and headache, while other clinical conditions were in the normal range. The patient managed to deliver a normal vaginal birth on April 2, 2020. The patient was in good clinical disease (80 scores on the Karnofsky scale) and could perform simple daily activities by herself. The only symptom was pain whenever the patient was trying to sit in a chair (pain score of 4 on a 1-10 numeric pain rating scale). The mother and baby were all in perfect health condition so that they could leave Puskesmas on April 3, 2020. The patient was prescribed mefenamic acid 500 mg three times a day for three days, two tablets of vitamin A once daily, 20 tablets of vitamin B complex, and iron supplementation. The patient attended her first postpartum (puerperium) care (day 7) on April 9, 2020 – her blood pressure was 100/60 mmHg and her body weight of 67 kg. The appearance of the perineal wound was wet and had not been unified yet, and the major complaint of pain on sitting (pain score of 3), but the patient managed to urinate and defecate normally. The patient was prescribed mefenamic acid 500 mg three times a day for another three days and vitamin B complex twice a day for seven days. ' 'patient's baseline knowledge of perineal wound care and infection prevention, as well as prophylaxis analgesic and antibiotic use for the specific case of perineal tears, and drug adherence was low (50% correct answers). Pharmacists re-educate the patient about the importance of drug adherence. The patient was 100% adherence to all medications, and the perineal wound had been drying without infection at her 2nd postnatal care visit (day 14) on April 16, 2020. Her blood pressure was slightly raised to 110/70 mmHg, and her body weight of 67 kg. The complete wound healing was achieved right after the 2nd visit (day-14) and persisted until 42 days postpartum.

Case 2.

Another 19 years-old primiparous women had never attended antenatal care at the 'Puskesmas' maternal clinic, but the patient delivered a normal vaginal birth at 3.37 AM April 3, 2020. The patient's weight was 65 kg, blood pressure was 110/70 mmHg, pulse = 50x/minute, and body temperature was 36° C. The patient got bleeding during labor, but otherwise was in a good clinical condition (80 scores on the Karnofsky scale) and could perform simple daily activities independently. The only symptom was pain. The mother and baby were all in perfect health condition so they could leave Puskesmas on April 4, 2020. The patient was prescribed mefenamic acid 500 mg three times a day, two tablets of vitamin A and iron tablets of 20 administered once daily. The patient attended her 1st postpartum (puerperium) care on April 11, 2020 – her blood pressure was 100/70 mmHg, a pulse of 50 times/ minute, and bodyweight of 65 kg. The appearance of the perineal wound was wet, and the patient complained of slight pain whenever the patient tried to get up from the chair (pain score of 2 on a 1-10 numeric pain rating scale), so the patient was prescribed mefenamic acid 500 mg three times a day for another three days. The patient's baseline knowledge of perineal wound care and infection prevention, as well as prophylaxis analgesic and antibiotics used for the particular case of perineal tears, was at a medium level (85% correct answers). However, the healthcare team counselled the patient due to non-compliance with antenatal care, and her low pulse could lead to a propensity to fall. Pharmacists educate the patient about the importance of drug adherence. The patient had 100% adherence to all medications, and the perineal tears were healed and dry at the 2nd puerperium care visit (day-14) on April 19, 2020. The ' 'patient's blood pressure was 110/70 mmHg. The complete wound healing persists until 42 days postpartum, with no sign of infection.

Case 3

A 30 years-old multiparous woman had been married for 12 years. The ' 'patient's past medical history

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was genital candidiasis, managed with ketoconazole 2 x 1 tablet. The patient's baseline knowledge of perineal wound care and infection prevention, as well as prophylaxis analgesic and antibiotics used for the certain case of perineal tears, was medium (85% correct answers). The patient attended a routine visit to the maternal clinic at Puskesmas on March 4, 2020, with no complaint, but her blood pressure was constantly around 100/60 mmHg (body weight was 60 kg). At the 32-33 weeks of the gestational period, the midwife found that her fetal presentation was not in a normal position, and the fetal pulse was 126 x/ minute. This high-risk pregnancy presented an unstable fetus position, and the patient had to be referred to the hospital. This fetal latitude position managed to be corrected weeks before the labor so that the patient was referred to Puskesmas and allowed to have a normal vaginal birth at term, and the patient managed to deliver a normal vaginal birth on April 22, 2020. The patient was in excellent clinical condition (90 scores on the Karnofsky scale) and could perform simple daily activities independently. There was no other complaint. The mother and baby were all in perfect health condition so they could leave Puskesmas on April 23, 2020. The patient was prescribed mefenamic acid 500 mg three times a day for three days, methyl ergometrine one tablet three times a day, two tablets of vitamin A, and iron tablets of 20 administered once daily. The patient attended her first postnatal (puerperium) care (day 5) on April 28, 2020 – her blood pressure was 100/70 mmHg, and her body weight of 57 kg. The perineal wound was healed and dry, with no sign of infection, and the complete wound persisted until 42 days postpartum the patient turned out to have 100% adherence to all medications.

Case 4

A multiparous woman aged 29 years old had never attended a routine antenatal care visit to the maternal clinic at Puskesmas, but the patient managed to deliver a normal vaginal birth on April 22, 2020. The healthcare team trained the patient to give exclusive breastfeeding to her baby, puerperium nutrition consumption, perineal wound care, and personal hygiene. Patient baseline knowledge of perineal wound care and infection prevention and prophylaxis analgesic and antibiotics use for some instances of perineal tears was medium (80% correct answers), so the pharmacist counselled the patient about the importance of drug adherence to the ' 'patient's health. The patient was in good clinical condition (80 scores on the Karnofsky scale) and could perform simple daily activities by herself. The mother and baby were all in perfect health condition so they could leave Puskesmas on April 23, 2020. The patient was prescribed mefenamic acid 500 mg three times a day for three days, two tablets of vitamin A and iron tablets of 20 administered once daily. The patient attended her 1st postpartum (puerperium) care on April 28, 2020 – her body weight was 55 kg, her blood pressure was 140/90 mmHg, and 15 minutes later, her blood pressure was 120/90 mmHg. The appearance of the perineal wound was wet, and the patient complained of slight pain (pain score of 3 on a 1-10 numeric pain rating scale), so the patient was prescribed mefenamic acid 500 mg three times a day for another three days. To avoid perineal wound infection, pharmacists re-educate the patient about the need for treatment adherence. Her perineal tears were healed and dried at the patient's second puerperium care visit on May 2, 2020. The patient took all these prescriptions exactly as prescribed. Her blood pressure was 120/90 mmHg at first, but 15 minutes later, it was 130/90 mmHg. The wound heals completely until 42 days after delivery, with no infection.

## DISCUSSION

This paper reports cases of pregnant women with low (case 1) to medium (case 2-4) knowledge about perineal wound care and infection prevention, prophylaxis analgesic and antibiotics use for some instances of perineal tears, well as the importance of drug adherence. Due to the slum environment of the ex-landfills, lack of personal hygiene, and some medical risk factors, such as hypotension (cases 1–3), bradycardia (case 1), fetal latitude position (case 3) that was turned into a normal presentation at term, non-compliance with antenatal care (cases 2 and 4), and possibly an undernourished state based on maternal body weight, the patients had an increased risk of infection (case 3 and 4). More than 75% of pregnant women are affected by perineal tears during labor. The risk factors for developing perineal infection were primiparous women, fetal weight (> 4000 grams), instrumental and post-term delivery, heredity pelvic floor dysfunction, and/ or connective tissue deficiency, and maternal birth position (Jansson *et al.*, 2020). The other risk factors for perineal tears were ≤ 20 years of age, Asian ethnicity, vaginal birth after cesarean section, epidural or oxytocin use, and midline episiotomy (Goh *et al*, 2018). Perineal tears could become the bacterial point of entry through the skin fissure. Perineal tears three-fold higher affected primiparous women than

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multiparous. Healthy multiparous women who have experienced more than one time of normal vaginal birth were able to practice the proper technique of delivering the baby. Thus, intact perineum after normal vaginal birth was three-fold higher in multiparous women (Smith *et al.*, 2013).

Theoretically, cases 3 and 4 of multiparous women would not be affected by the wound tears and pain compared to cases 1 and 2 of primiparous ones. These were unique cases in which seemed parietal status did not serve as a risk factor for the perineal 'tears' incidence, for all four of these pregnant women experienced perineal tears pain, which ranged from "no pain" scored 0 to "pain" scored 3 on numerical pain rating scales. They all managed to have a normal vaginal birth, assisted by the physician and midwives. The woman in case 3 was the only person with no pain ' 'or any other complaint – even though her baby's latitude position complicated the normal vaginal birth. This temporary latitude position would be a fetal risk factor for perineal tears and a cesarean section indication. (Jansson *et al*, 2020; Goh *et al*, 2018). At the end of pregnancy, the latitude position was turned into a normal presentation so that the mother could give a vaginal birth safely. Perineal tears of this woman of case 3 were healed and dry at her 1st attendance of puerperium care (7 days after the birth). The patient had no pain according to the numeric pain rating scale nor any complaint. The numeric pain rating scale is a subjective measurement of an "awful sense or hurt of the body", thus, this tool could not serve as an objective measurement (Haefeli and Elfering, 20006). The woman in case 3 had a complicated pregnancy due to fetal shoulder dystocia during the last trimester. This fetal position was adjusted during labor and delivery, allowing the patient to have a normal vaginal birth safely with the physician's intervention at Puskesmas.

Emerging clinical evidence from a systematic review showed no statistical differences in the incidence of infection prevention with and without routine prophylaxis antibiotics after normal vaginal birth (Bonet *et al*, 2017; Tandon and Dalal, 2018). A Cochrane intervention review concluded that antibiotics are not a substitute for infection prevention and control measures around childbirth and the postpartum period (Bonet *et al*., 2017). Thus, antibiotics and analgesic prophylaxis should be administered to prevent pain and 3rd or 4th degree of perineal wound infection, while 1st and 2nd degrees of the perineal wound were unlikely to develop an infection in healthy pregnant women (RCOG 2015; WHO 2015). All four cases of these pregnant women were prescribed mefenamic acid 500 mg three times a day for 3 days after giving birth, 2 of them got a pain score of 2 on a 1-10 numeric pain rating scale, and 1 of them had a pain scale of 3. Mefenamic acid 500 mg three times a day was prescribed for another three days on their 1st attend to the maternal clinic – which was seven days after giving birth. Despite 'patients' knowledge about preventing perineal wound infection without antibiotics and maternal supplementation administration being low to medium levels before normal vaginal delivery, pharmacist re-education activities seemed to improve 'patients' knowledge and drug-taking behavior. They adhered to medicines regimens and had no more prolonged pain on their 2nd attend the maternal clinic 14 days after the birth until the perineal tears completely healed without sign of infection on day 42.

The risk factors of developing bacterial infection were quite high among these four pregnant housewives due to the slum environment of the ex-landfills near their houses, lack of knowledge about personal hygiene, and perineal wound care. In the last trimester, pregnant women should attend the maternal clinic twice weekly, then weekly visits in the previous 2 weeks of pregnancy. The postpartum (puerperium) care should have been given to them at Puskesmas maternal clinic in 3 days intervals after giving birth. Instead, it had to be adjusted to days-7, days-14, and days-42 visits. The longer visit interval, combined with online monitoring through social media applications and telephone calls, theoretically leads to a higher incidence of perineal tears infections for these low education and social, and economic levels of the mother who just gave birth.

The strength of this research was the solid health care team that performed a counselling program that managed to overcome these obstacles by:

1. Identifying the problem of lack of knowledge about infection prevention and drug use based on questionnaires and interviews as early as an antenatal visit to the Puskesmas. Pharmacists could successfully overcome these problems by performing close supervision and routine-repeated education regarding drug adherence.

2. Motivating pregnant women to apply knowledge and training on perineal wound care who received stitches, take medication, and maintain postpartum nutrition intake during counselling into infection prevention behavior and medication adherence due to the lack of" face-to-face visits" during the pandemic; this information was acquired based on maternal self-

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report via telephone and WhatsApp conversation during the postpartum period.

3. Reducing the risk of infection. There was no single incidence of suture wound infection based on doctors' and midwives' observation of maternal perineum condition in every Puskesmas visit during labor and puerperium.

Since May 2021, all pregnant women have been referred to the hospital for a compulsory RT-PCR test of SARS CoV-2 RNA materials and more comprehensive perinatal care due to the immediate and massive escalating Covid-19 pandemic in Indonesia. This situation led to the fundamental limitation of this research, which was (a) the outcome monitoring method had to be changed to more extended interval visits, (b) the delivery method of the counselling program, had to rely on the social media application and telephone calls could lead to a -sub-optimal outcome, and (c) there was only shortlisted number of patients included in this research, which might not be adequate to report it as a case-control study.

## CONCLUSION

It can be concluded that 2$^{nd}$-degree perineal tears infection following normal vaginal birth could be prevented without antibiotics. The effectiveness of the counselling program's delivery method, which relies on social media applications and telephone calls, must be validated.

We recommend to performed double-blind, randomized controlled trials for future research for more robust clinical evidence. It is strongly suggested to control the maternal nutritional states, potentially interfering with the maternal ability to heal the perineal wound.

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## AUTHOR CONTRIBUTIONS

Conceptualization, F.O.H.P., A.P.; Software, A.P.; Methodology, F.O.H.P., A.P.; Validation, F.O.H.P.; Formal Analysis, A.P.; Investigation, L.H.; Resources, L.H.; Data Curation, A.P.; Writing - Original Draft, L.H.; Writing - Review & Editing, L.H., A.P.; Visualization, L.H.; Supervision, F.O.H.P., A.P.; Project Administration, F.O.H.P., A.P.; Funding Acquisition, L.H.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Assessment of Antibiotic Use in ICU Patients with Pneumonia Using ATC/DDD as a Quantitative Analysis Method

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***Abstract***

***Background:*** *Pneumonia is an infection of the lung tissue which is mainly caused by bacteria. High utilization and inappropriate use of antibiotics increase resistant bacteria, morbidity, mortality, and treatment cost. Quantitative evaluation becomes one of the indicators to assess the use of antibiotics which is one indicator of the quality of the antimicrobial resistance control program.* ***Objective:*** *This study aimed to evaluate the use of antibiotics an effort to increase the rationality of the use of antibiotics.* ***Methods:*** *This study was a retrospective observational study with a sampling method of time-limited sampling in Universitas Airlangga hospital from January until December 2019. Quantitatively using Anatomical Therapeutic Chemical (ATC)/Defined Daily Dose (DDD).* ***Result:*** *The samples obtained were 68 severe pneumonia patients who met the inclusion criteria. From the result of the study, there were 13 types of antibiotics used for pneumonia therapy, and the three most used were Levofloxacin, Ceftriaxone, and Meropenem, with a total of all antibiotics 73.64 DDD/100 patients-days. Most useful is parenteral Levofloxacin at 21.92 DDD/100 patient-days, Ceftriaxone at 20.45 DDD/100 patient-days and Meropenem at 14.29 DDD/100 patient-days.* ***Conclusion:*** *The DDD value indicates high antibiotic usage, but high antibiotic use does not imply unreasonable drug use, so we must undertake a qualitative review of antibiotic use.*

***Keywords****: pneumonia, severe pneumonia, ICU, antibiotics, ATC/DDD*

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## INTRODUCTION

Pneumonia is a pulmonary inflammation caused by various microorganisms (PPDI, 2014), mainly caused by *Streptococcus pneumoniae (pneumococcus), Staphylococcus aureus, Streptococcus Group A, Klebsiella pneumoniae, Haemophilus influenzae* (Pahal et al., 2021), and atypical bacteria such as *Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila* (Stamm & Stankewicz, 2021). In a study of 7749 Community-Acquired Pneumonia (CAP) patients, 23% were admitted to the intensive care unit (ICU), and the mortality rate was 47% in 1 year (Cavallazzi et al., 2020). A patient has severe CAP if he has at least one of two major or at least three minor criteria, according to criteria developed and validated by the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS). Septic shock and respiratory failure are major criteria. In contrast, minor criteria include a respiratory rate/frequency of breath of fewer than 30 breaths per minute, a PaO2/FIO2 ratio (ratio of arterial oxygen partial pressure to fractional inspired oxygen) of less than 250 mmHg, multilocus infiltration on thoracic photos, decreased awareness or disorientation, uremia, and hypotension (Metlay *et al.*, 2019). Antibiotics are the most common treatment for bacterial pneumonia (Farida *et al*., 2017). Inappropriate antibiotic use leads to antibiotic resistance, the most serious concern in treating illnesses like pneumonia (Pratama *et al*., 2019). The World Health Organization (WHO) is working to reduce antibiotic resistance internationally, including developing a global action plan that increases antibiotic usage responsibility and assesses antibiotic use (Pratama *et al*., 2019). The Anatomical Therapeutic Chemical (ATC)/Defined Daily Dose (DDD) technique can be used to determine the kind quantitatively and amount of antibiotics taken, while the Gyssens method can be used to assess antibiotic use accuracy (Metley *et al.,* 2019; Farida *et a.l,* 2017) qualitatively. The WHO approved the ATC/DDD system as the international measurement standard for drug use research (Pratama *et al*., 2019). With the high frequency of severe pneumonia and the rising incidence of antibiotic resistance, the primary treatment for severe pneumonia, more study is needed to evaluate the use of antibiotics in severe pneumonia patients using ATC/DDD to enhance antibiotic utilization rationale.

## MATERIALS AND METHODS

### Materials

Research materials in the form of Medical Records (RM) of patients with pneumonia diagnoses who undergo hospitalization and get antibiotic therapy in the Intensive Care Unit (ICU) of Universitas Airlangga Surabaya Hospital in the period January-December 2019 that meet the criteria of inclusion

### Methods

#### Study population and design

The observational study used data from pneumonia patients in the intensive care unit's medical records. The study included all patients with pneumonia admitted to the Universitas Airlangga Surabaya Hospital's ICU and received antibiotic therapy between January and December 2019. The inclusion criteria were Pneumonia patients over 18 who had been diagnosed with CAP or HAP and were being treated with antibiotics. Patients diagnosed with infections for which antibiotics were not administered, patients discharged from the hospital on their request, and patients who died were also excluded.

#### Data collection instrument

Research materials in the form of Medical Records of patients, with data collected in the form of Medical Record numbers, Patient Identity (name, gender, age, date of first treatment and completion of therapy), patient disease history, complaints and diagnosis, clinical data, laboratory data, microbiology data and antibiotic therapy, which includes the type of antibiotic, route, dosage, interval, and a length of stay (LOS). Then the data is recorded on the Data Collection Sheet (DCS) and the master table, which is the research instrument. The sampling technique uses time-limited sampling, so all inclusion samples are in this study.

#### Data analysis

Data analysis included sex, age, type of pneumonia, and comorbidity; analysis of bacterial patterns that cause severe pneumonia and bacterial sensitivity to various kinds of antibiotics based on the results of microbiological examinations of patients, and analysis of antibiotic use patterns in severe pneumonia patients, including type, route, dose, and interval of administration. ATC/DDD with DDD/100 patient days measurement unit was used to investigate antibiotic use quantitatively. Calculation of antibiotic consumption :

$$\text{DDD/100 days of hospitalization} = \frac{total\ antibotics\ sold\ in\ a\ year\ (grams)}{WHO\ DDD\ Standard\ (gram)}\ x\ \frac{100}{population\ x\ 365}$$

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The quantity of antibiotic use can also be expressed in DDD / 100 patient days using calculation using the formula (Kemenkes RI, 2011).

$$\text{DDD/100 patient days} = \frac{\text{total dose of antibiotics used by the patient (gram)}}{\text{WHO DDD Standard (gram)}} \; x \; \frac{100}{\text{Total Length Of Stay}}$$

## RESULT

### Demographic variables

Demographic variables include gender, age, type of pneumonia, symptoms, and accompanying disease. The results of the gender distribution showed that the number of male patients with severe pneumonia was more than female patients, which was 35 out of 68 patients (52%). In Europe, the incidence of CAP in men is higher than in women. Lifestyle is a high-risk factor for pneumonia, including smoking, alcohol, low BMI and malnutrition, household arrangements, and poor dental hygiene. In addition, the presence of immunocompetent and immunocompromised also increases the risk of pneumonia (Cillóniz *et al.,* 2017). Age distribution indicates dominated by the age group > 65 years. Age, especially 65 years and above, is one of the risk factors for pneumonia and clinical manifestations that generally appear severe (Barbara G. *et al.*, 2015; Torres *et al.*, 2013). The most common type of pneumonia was CAP, with 55 out of 68 patients (81%). Both CAP and HAP can develop into severe infections when an uncontrolled inflammatory reaction is caused by causative pathogenic factors and/or individual factors such as increasing age and comorbidities (Waterer, Grant W. and Wunderink, 2005). Clinical symptoms that are often found in cases of severe pneumonia are fever, cough accompanied by the production of sputum purulent, dyspnea (shortness of breath), and chest pain. In addition, there can also be changes in mental status/disorders of consciousness (Wendy I. & Thomas J., 2013). Based on data from 22 patients, shortness of breath is a symptom experienced by all patients. The characteristics of the patients studied are in Table 1.

### Number of days hospitalized/length of stay

The number of days a patient spends in the hospital is measured from when they are diagnosed with pneumonia until they are diagnosed with pneumonia after leaving the hospital. The total length of stay for all patients was 616 days, with an average length of stay of 9.0 days, indicating that patients with severe pneumonia in RSUA Surabaya in 2019 spent 9 days in the hospital (table.2). Patients with bacterial infections are often admitted to the hospital for 7–10 days. However, this might be extended depending on comorbidities, consequences, and the severity of the infection (Menéndez *et al*., 2001).

**Table 1.** Characteristics of pneumonia patients at RSUA Surabaya period January-December 2019

| Demographic characteristics | | Number | Percentage (%) |
|---|---|---|---|
| **Gender** | Male | 35 | 52 |
| | Female | 33 | 48 |
| **Age (years)** | 17-25 | 1 | 2 |
| | 26-30 | 0 | 0 |
| | 36-45 | 3 | 4 |
| | 46-55 | 10 | 15 |
| | 56-65 | 19 | 28 |
| | >65 | 35 | 51 |
| **Type of pneumonia** | CAP | 55 | 81 |
| | HAP | 13 | 19 |
| **Clinical Symptoms*** | Dyspnea | 22 | 38.6 |
| | Fever | 7 | 12.3 |
| | cough | 9 | 15.8 |
| | Productive cough | 7 | 12.3 |
| | chest pain | 2 | 3.5 |
| | Delirium | 10 | 17.5 |

*Data from 22 patients; One patient may experience more than one clinical symptom.

**Table 2.** Number of hospitalization days of severe pneumonia patients at RSUA Surabaya period January-December 2019

| Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Amount |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| The number of patients | 12 | 10 | 7 | 6 | 7 | 4 | 2 | 4 | 3 | 3 | 6 | 4 | 68 |
| LOS | 105 | 64 | 102 | 63 | 82 | 34 | 19 | 18 | 8 | 50 | 46 | 25 | 616 |
| Average LOS | 8.8 | 6.4 | 14.6 | 10.5 | 11.7 | 8.5 | 9.5 | 4.5 | 2.7 | 16.7 | 7.7 | 6.2 | 9.0 |

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**Quantitative analysis of antibiotic use in severe pneumonia patients**

The results showed that the total use of antibiotics in severe pneumonia patients who underwent hospitalization at ICU RSUA Surabaya in January 2019 was 73.64 DDD/100 patient-days (table 3.). Of the 13 antibiotics prescribed for severe pneumonia therapy, parenteral Levofloxacin is the most widely used antibiotic, with total use of 21.92 DDD/100 patient-days which can be interpreted that in 100 days of hospitalization at RSUA Surabaya, there are 21-22 severe pneumonia patients who get Levofloxacin parenteral therapy according to the WHO's defined daily dose of 0.5 grams per day. The second most antibiotic use is Ceftriaxone, with total use of 20.45 DDD/100 patient-days. The third most antibiotic is meropenem, with total use of 14.29 DDD/100 patient days.

Furthermore, the use of antibiotics in a row followed by parenteral Moxifloacacy (5.20 DDD/100 patient-days); oral Levofloxacin (2.76 DDD/100 patient-days); Amikacin (2.56 DDD/100 patient-days); Ceftazidime (2.31 DDD/100 patient-days); Trimethoprim-Sulfamethoxazole oral (1.87 DDD/100 patient-days); Cefazolin (0.54 DDD/100 patient-days); oral Erythromycin (0.49 DDD/100 patient-days); Gentamicin (0.43 DDD/100 patient-days). The least used of antibiotics is Cefoperazone -Sulbactam and Vancomycin with the same amount of use of 0.41 DDD/100 patient-days (Figure 1).

**DISCUSSION**

On average, severe pneumonia patients at RSUA Surabaya undergo nine-day hospitalisation. In general, patients who have a bacterial infection undergo hospitalization for 7-10 days but can be extended depending on the accompanying disease suffered, complications experienced, and the severity of the infection (Menéndez *et al.*, 2001). The use of antibiotics in severe pneumonia patients in RSUA Surabaya is still reasonably high (73.64 DDD/100 patient-days). This has the potential to trigger irrational use of antibiotics, so it is expected that in the future, this data can be a consideration in the prescribing of antibiotics in hospitals. The DDD/100 patient-days are linear with the rate of antibiotic use within 100 days of treatment, which means the greater the DDD/100 patient-days value, the higher the rate of antibiotic use (Sari & Safitri, 2016). Compared to 98 studies conducted in hospitals in Germany that showed total antibiotic use in sepsis and pneumonia patients of 51.0 DDD/100 patient-days (Scholze *et al.*, 2015)

**Table 3.** Results of quantitative evaluation of antibiotic use in severe pneumonia patients in RSUA Surabaya period January-December 2019 with ATC / DDD method

| Class antibiotics | Code ATC | Name antibiotics | Route* | Total Use (gram) | DDD Standard WHO (gram) | Total DDD Use | LoS (day) | DDD/100 patientdays | DDD/100 patient-days Group |
|---|---|---|---|---|---|---|---|---|---|
| Cephalosporins | J01DB04 | Cefazolin | P | 10 | 3 | 3.33 | 616 | 0.54 | 23.71 |
| | J01DD02 | Ceftazidime | P | 57 | 4 | 14.25 | | 2.31 | |
| | J01DD04 | Ceftriaxone | P | 252 | 2 | 126 | | 20.45 | |
| | J01DD62 | Cefoperazone - Sulbactam | P | 10 | 4 | 2.5 | | 0.41 | |
| Carbapenem | J01DH02 | Meropenem | P | 264 | 3 | 88 | | 14.29 | 14.29 |
| Sulfonamide | J01EE01 | Trimethoprim-Sulfamethoxazole | O | 22.08 | 1.92 | 11.5 | | 1.87 | 1.87 |
| Macrolides | J01FA01 | Erythromycin | O | 3 | 1 | 3 | | 0.49 | 0.49 |
| Aminoglycosides | J01GB03 | Gentamicin | P | 0.64 | 0.24 | 2.67 | | 0.43 | 2.99 |
| | J01GB06 | Amikacin | P | 15.75 | 1 | 15.75 | | 2.56 | |
| Fluoroquinolones | J01MA02 | Moxifloxacin | P | 12.8 | 0.4 | 32 | | 5.20 | 29.88 |
| | J01MA12 | Levofloxacin | P | 67.5 | 0.5 | 135 | | 21.92 | |
| | | | O | 8.5 | 0.5 | 17 | | 2.76 | |
| Glycopeptide | J01XA01 | Vancomycin | P | 5 | 2 | 2.5 | | 0.41 | 0.41 |
| Total DDD/100 patient-days | | | | | | | | | 73.64 |

*O= Oral, P= Parenteral

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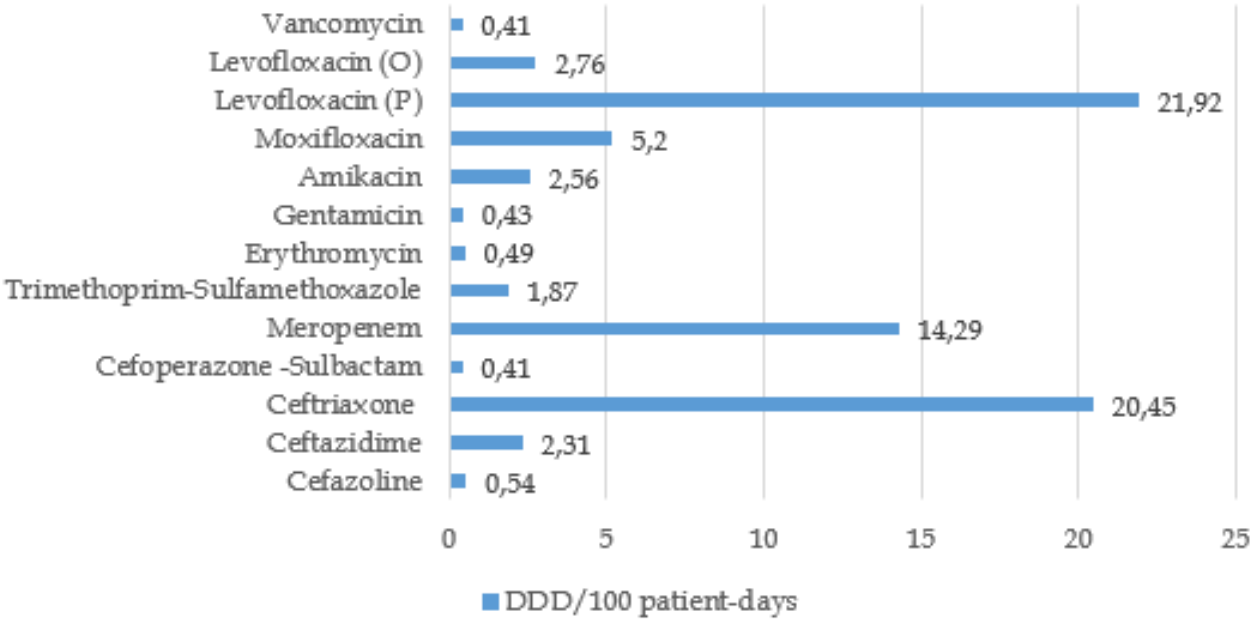


**Figure 1.** Quantity of antibiotic use by type in the patient severe pneumonia at RSUA Surabaya for the period January-December 2019

Parenteral Levofloxacin is the most widely used antibiotic in RSUA Surabaya. Similarly, it was found in studies conducted at Dr Iskak Tulungagung Hospital and Dr Moewardi Surakarta Hospital, with the number of Levofloxacin used for consecutive indications of pneumonia reaching 40.14 DDD/100 patient-days and 53.88 DDD/100 patient-days (Ilmi *et al.*, 2020; Muhammad & Mutmainah, 2018). Levofloxacin is among the most widely prescribed types of antibiotics for pneumonia therapy because it is a respiratory fluoroquinolone-class antibiotic that is effective in overcoming upper and lower airway infections with high activity against gram-positive bacteria and atypical bacteria cause pneumonia (Daniel H & Lisa G, 2015; Izadi *et al.*, 2019). Various studies have reported clinical cure rates of pneumonia patients who get l Levofloxacin ranging from 94-98% and, in severe cases, by 87% (Ball, 2003). However, it should be noted that Levofloxacin is a broad-spectrum antibiotic susceptible to the incidence of resistance when used excessively.

Ceftriaxone is one of the third-generation cephalosporins most active in penicillin-resistant *pneumococci* strains, so it is widely used as empirical therapy for severe infections caused by such pathogens (Daniel H & Lisa G, 2015). However, there is a study that proves an association between the increased use of third-generation cephalosporin groups and the increased incidence of ESBL-producing bacteria (Extended Spectrum Beta-Lactamase) (Urbánek *et al.*, 2007). Also the use of this group needs special attention. Meropenem of the carbapenem group is active against various aerobic and anaerobic bacteria, including penicillin-resistant *pneumococci* strains such as Ceftriaxone, and has shown promising effectiveness in severe pneumonia therapy (Baldwin et al., 2008).

Rational use of antibiotics in hospitals requires a restrictive policy in their application. Antibiotics are divided into two groups: those that are free to be prescribed by any clinician (non-restricted) and those that are restricted (restricted and reserved) (Ball, 2003).. For example, Vancomycin, which was classified as a reserved antibiotic in this study due to its low number of uses, must be prescribed with the approval of the Antibiotic Resistance Control Program (PPRA) at Universitas Airlangga Hospital. In addition to these restrictions, a policy of financing and procurement of antibiotics supports the rational implementation of antibiotic use in hospitals (Kemenkes RI, 2015).

There have been similar studies on antibiotics in pneumonia patients, but they showed different results. These differences can be caused by differences in local germ patterns and/or the severity of infection. In addition, the results of the culture and the clinical circumstances of the patients are taken into account while choosing a treatment. In a study conducted by Gayatri and Bestari (2017) at RSUPN Dr. Cipto Mangunkusumo Jakarta obtained data that azithromycin is the most widely used antibiotic by pneumonia patients, with total use of 62.5 DDD/100 patient days (Gayatri & Bestari, 2017).

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Evaluation of antibiotic use in hospitals both quantitatively and qualitatively needs to be done periodically to be able to know trends or changes in their use over time and become the material for assessing the success of PPRA in hospitals, namely by looking at the number or absence of improvement in the quantity and quality of antibiotic use. In terms of amount, the improvement in question is reducing the number and type of antibiotics used as empirical and definitive therapies (Kemenkes RI, 2015). Changes in the use of antibiotics kinds can also be used as a reference in the procurement of further drugs (Hasrianna *et al.*, 2015).

## CONCLUSION

One method is to assess the use of antibiotics. ATC/DDD is the approach employed. According to the findings, the total use of antibiotics in 68 patients with severe pneumonia who were hospitalized in the ICU at RSUA Surabaya from January to December 2019 was 73.64 DDD/100 patient days. With 21.92 DDD/100 patient-days total use, parenteral Levofloxacin is the most commonly used antibiotic Ceftriaxone (20.45 DDD/100 patient-days) and meropenem (14.29 DDD/100 patient-days). Patient data from fully recorded medical records is limited to 22 of 68 patients. Hence some discussion points only include information from those 22 individuals. The DDD value suggests a high use of antibiotics, but increased usage of antibiotics does not necessarily indicate irrational use of antibiotics. Thus, a qualitative evaluation of antibiotic use is required.

## CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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## AUTHOR CONTRIBUTIONS

Conceptualization, M.R.A., A.N.R., T.A.; Software, M.R.A.; Methodology, M.R.A., A.N.R., T.A.; Validation, M.R.A., A.N.R.; Formal Analysis, M.R.A., S.M.N., Z.N.; Investigation, M.R.A., S.M.N.; Resources, M.R.A., S.M.N.; Data Curation, M.R.A., A.N.R., T.A.; Writing - Original Draft, M.R.A., S.M.N., Z.N.; Writing - Review & Editing, M.R.A., S.M.N., Z.N.; Visualization, M.R.A.; Supervision, M.R.A.; Project Administration, M.R.A.; Funding Acquisition, M.R.A.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Formulation and Characterization of Carbamazepine Chitosan Nanoparticle

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***Abstract***

***Background****: Carbamazepine is an antiepileptic drug used to treat trigeminal neuralgia and pain associated with neurological disorders. The drug belongs to class II of the Biopharmaceutical Classification System (BCS), which has low solubility. Hence, dissolution is a rate-limiting step.* ***Objective****: This study aimed to determine the best formula for carbamazepine nanoparticles based on physical characteristics and determine the effect of chitosan and Na-TPP concentration variation on nanoparticle characterization.* ***Methods****: The carbamazepine chitosan nanoparticles were prepared using ionic gelation method with a concentration of 0.1% w/v carbamazepine and the ratio of chitosan and Na-TPP concentrations of 0.2%:0.1% (F1), 0.2%:0.2% (F2), and 0.3%:0.1% w/v (F3). The parameters evaluated included particle size, polydispersity index, zeta potential, particle morphology, and entrapment efficiency. Statistical analysis was conducted on the evaluation data using One Way ANOVA.* ***Results****: The results showed that the effect of increasing the concentration of chitosan reduced particle size ($p<0.05$), increased zeta potential ($p<0.05$), and had no effect on the value of entrapment efficiency ($p>0.05$). Furthermore, F3 had a particle size of 169.8±13.71 nm with a polydispersity index of 0.378±0.02, the zeta potential of +28.80±2.44 mV, entrapment efficiency of 84.3±7.50%, and spheric particle morphology which was measured using Transmission Electron Microscope (TEM).* ***Conclusion****: Therefore, F3 with the ratio of chitosan and Na-TPP concentrations of 0.3%:0.1% was the formula that provided the best characteristics of chitosan carbamazepine nanoparticles.*

***Keywords****: carbamazepine, chitosan, epilepsy, nanoparticle*

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## INTRODUCTION

Carbamazepine is an effective anticonvulsant drug that controls the grand mal and psychomotor convulsions (Zafrul & Halim, 2014). However, the drug's weakness is that it is practically insoluble in water (113 µg/mL at 25$^{o}$C) (Sethia & Squillante, 2002). Therefore, carbamazepine is categorized in the Biopharmaceutical Classification System (BCS) class II, which indicates a slow dissolution rate and high membrane permeability (Nair et al., 2012). The factor that causes its practically non-dissolved nature in water is due to the existence of an aromatic group, double bond, and hydrophobic long hydrocarbon chain (Wardiyah, 2016). Meanwhile, a drug that is difficult to dissolve in water, especially an oral drug, has its absorption controlled by the dissolution rate in the gastrointestinal tract (Nair et al., 2012).

The use of nanoparticle technology is a promising approach to increasing carbamazepine solubility. Moreover, reducing the particle size into the nanometer range increases the contact width of the compound surface with the medium, increasing solubility, dissolution rate, and drug permeability due to the increasing penetration capacity into the cell (Martien et al., 2012).

In this study, chitosan, a chitin derivative polysaccharide was used as the polymer. It is often used as a polymer in the formation of nanoparticles due to its nontoxic, mucoadhesive, biodegradable, biocompatible, and hydrophilic nature. The amino group in chitosan creates uniqueness in its structural aspect compared to other polysaccharides, which gives it a cationic character. This character incurs a strong electrostatic interaction between chitosan and anionic drug. Therefore, its usage is very good within the drug transport system (Harahap, 2012).

One method to synthesize chitosan nanoparticles is through ionic gelation, which reduces the particle to nanometer size. The principle is based on the electrostatic interaction between the positive amino group (-$NH_2$) in chitosan and the negative charge group of the polyanion, namely natrium tripolyphosphate (Na-TPP) (Harahap, 2012). Moreover, Na-TPP has superiorities as the cross-linking material is not poisonous, affordable in price, stable, and has more negative charges than other polyanions, which causes stronger interaction (Marrisa, 2017). The prevalence of this ionic gelation method, among other methods, includes its simplicity, ease to obtain, and no heating involved in the process (Irianto & Muljanah, 2011). Meanwhile, azithromycin with a carbonyl group similar to carbamazepine is successfully bound with an amino group in chitosan to form azithromycin chitosan nanoparticles. These nanoparticles have a size of 172.9 nm (PDI 0.265) and a zeta potential of +41.3 mV, which is obtained by mixing 0.1% of chitosan and 0.1% of Na-TPP (Mannuela, 2016). Therefore, carbamazepine has the potential to be formulated into nanoparticles. A previous study by Mardliyati et al., (2012) formulated nanoparticles without active substance and the particle size obtained was less than 100 nm with chitosan concentrations Na-TPP of 0.2%:0.1%. Hence, the ratio of concentrations of chitosan and Na-TPP will determine the success of nanoparticles formed by the ionic gelation method.

In this study, the formulation of carbamazepine nanoparticles was carried out using the ionic gelation method by varying the concentrations of chitosan and Na-TPP. The best formula was obtained by examining the characteristic results, which include the smallest particle size below 1000 nm, polydispersity index less than 0.5, biggest zeta potential of approximately ±30 mV, and highest adsorption efficiency evaluation above 60%.

## MATERIALS AND METHODS

### Materials

The materials used include carbamazepine pharmaceutical grade (MCF-Zhejiang Jiu, China), pro-analyzed (p.a) grade low molecular weight chitosan with a deacetylation degree of 83% and viscosity of 108 Cps (Sigma-Aldrich-Elo Karsa Utama), Na-TPP p.a (Sigma-Aldrich-Elo Karsa Utama), aquadest, acetate acid glacial (Merck), aqua demineralization, and methanol p.a (Merck).

### Tools

The tools used include Particle Size Analyzer (PSA) (Malvern Instrument) and UV spectrophotometer (Shimadzu UV-1900).

### Methods

#### Preparation of chitosan and Na-TPP solutions

The chitosan solutions were prepared by dissolving 50 mg or 75 mg of chitosan in 25 mL of 0.5% v/v acid acetate solution using the magnetic stirrer (LabTech) for 10 minutes to obtain two different concentrations of chitosan, which is 0.2% b/v and 0.3% b/v. Acetic acid 0,5% solution was prepared by diluting 0.5 mL acetic acid glacial in approximately 100.0 mL of aquadest (Iswandana et al., 2013). Furthermore, the Na-TPP solution concentrations of 0.1% b/v and 0.2% b/v were

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prepared by dissolving each of the 10 mg and 20 mg of Na-TPP in a 10mL measuring flask of aquadest (Iswandana et al., 2013).

**Preparation of carbamazepine chitosan nanoparticles**

Carbamazepine of 12.5 mg was dissolved with 12.5 mL methanol to obtain a 1 mg/mL concentration. It was added to 25 mL chitosan solution and stirred at 350 rpm using a magnetic stirrer (LabTech). A total of 5 mL Na-TPP was dripped into the mixture of chitosan-carbamazepine at room temperature of (±25°C) and stirred for 10 minutes at 350 rpm using the magnetic stirrer (LabTech) (Mannuela, 2016), to obtain a total dispersion volume of 42.5 mL nanoparticles. Meanwhile, the formula of carbamazepine chitosan nanoparticles is shown in Table 1.

The carbamazepine nanoparticle suspensions were then subjected to evaporation using a water bath at a temperature of 40ºC for 24 hours to eliminate the methanol. Furthermore, freeze-drying (VirTis benchtop K 2KBTXL-75) was conducted to obtain the powder of carbamazepine chitosan nanoparticles (Mannuela, 2016). Before the freeze-drying, the transmittance percentage was measured using a UV spectrophotometer (Shimadzu UV-1900) at a wavelength of 650 nm and a blank containing distilled water to ensure the clear solution had a particle of nanometer size. When the transmittance was above 90%, a particle size within the nanometer range was achieved (Huda & Wahyuningsih, 2016).

**Characterization of carbamazepine chitosan nanoparticles**

**Particle size and polydispersity index**

The particle size was analyzed with Particle Size Analyzer (PSA) (Malvern Instrument) using the technique of Dynamic Light Scattering (DLS) (Rasmussen et al., 2020). The parameters analyzed were average particle diameter and polydispersity index. Subsequently, the sample of nanoparticle powder was diluted with deionized aqua (Jazayeri et al., 2016) using the ratio of 4:5 b/v before being analyzed.

**Zeta potential**

Zeta potential was measured based on Laser Doppler Electrophoresis (LDE) method using the Zeta Sizer tool similar to Particle Size Analyzer (Malvern Instrument) (Rasmussen et al., 2020). Also, the potential can be measured by determining the particle speed and charge in the electricity field. The analysis of Zeta potential was carried out by dissolving the powders of carbamazepine chitosan with deionized aqua (Jazayeri et al., 2016) under the ratio of 4:5.

**Morphology**

The particle morphology was determined using Transmission Electron Microscopy (TEM) (JEM-1400) by dripping the sample of 10 µL into the grid and holding it for 1 minute. The residue volume within the grid was absorbed with filter paper and dried for 30 minutes before it was finally observed (Wulandari & Nugroho, 2020). This assay was done on the best formulation of carbamazepine nanoparticles (F3).

**Entrapment efficiency**

The entrapment efficiency was determined by measuring the concentration of free carbamazepine in the dispersion medium. Subsequently, 10 mg of the carbamazepine nanoparticle powder was dispersed into the 10 mL methanol and kept overnight before being centrifuged at 5000 rpm for 10 minutes. The supernatant was further filtered with a 0.2 µm membrane filter and analyzed with a UV spectrophotometer (Shimadzu UV-1900) at 280.4 nm, and the result obtained was considered a free drug (Arya et al., 2015). Whilst the total drug was calculated as the initial amount of carbamazepine added in the formula (12.5 mg). The percentage (%) of entrapment efficiency is calculated using Equation 1 below.

$$\text{Entrament Efficiency} = \frac{\text{total drug} - \text{Free drug}}{\text{Total drug}} \text{ x } 100\% \quad \ldots\ldots \quad (1)$$

**Table 1.** Formula of carbamazepine chitosan nanoparticles

| Formula (F) | Carbamazepine (% b/v) | Chitosan (% b/v) | Na-TPP (% b/v) | The actual amount in formulation (mg) | | Weight ratio Chitosan: Na-TPP (w/w) |
|---|---|---|---|---|---|---|
| | | | | Chitosan | Na-TPP | |
| 1 | 0.1 | 0.2 | 0.1 | 5 | 0.5 | 10:1 |
| 2 | 0.1 | 0.2 | 0.2 | 5 | 1 | 5:1 |
| 3 | 0.1 | 0.3 | 0.1 | 7.5 | 0.5 | 15:1 |

Volume ratio of Chitosan : Na-TPP is 5:1

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**Data analysis**

The data analysis was based on the characterization results of carbamazepine chitosan nanoparticles of F1, F2, and F3. The formula which fitting the ideal characteristic of nanoparticles, such as having the smallest particle size (<1000 nm), polydispersity index of less than 0.5, the zeta potential of approximately ±30 mV, the highest entrapment efficiency value above 60% and a spherical morphology was considered as best formula in this study. (Mannuela, 2016). Furthermore, the characterization results in particle size, zeta potential, and entrapment efficiency of all formulas were analyzed statistically using SPSS 25.0 under normality and homogeneity tests to evaluate the effect of concentration variation between chitosan and Na-TPP in the nanoparticle formation. When the data were homogenously ($p \geqslant 0.05$) and normally distributed ($p \geqslant 0.05$), the statistics test was continued with One Way ANOVA and Post Hoc, and when the data were not, Kruskal-Wallis and Mann Whitney non-parametric tests are to be conducted. Meanwhile, the data have significantly different meaning in statistics when parametric or non-parametric tests gives 0.05.

**RESULTS AND DISCUSSION**

The first observation was carried out against the maximum concentration of carbamazepine which can be dissolved in methanol. Moreover, all materials, such as chitosan, Na-TPP, and carbamazepine, must dissolve in their respective solvents (Wu et al., 2005). The observation of the solubility of carbamazepine in methanol was carried out by visual selection to produce a clear solution without any hovering particle. Based on the observation, the maximum concentration that sufficiently dissolved carbamazepine within methanol was the carbamazepine concentration of 0.1% b/v. Also, low viscosity chitosan is a weak base (pKa of 6.5) therefore, it was well-dissolved in an acid solution having a pH value of less than 6 (Mannuela, 2016). In the acidic pH, the amino group of chitosan ($-NH_2$) is protonated, causing chitosan to be a polycationic ($-NH_3^+$) molecule that can dissolve in water and interact through ionic bonding with the anionic drug. However, when the pH was more than 6, the amino group in chitosan was deprotonated and unable to form a crosslinking with other counter ions (Harahap, 2012). In addition, Na-TPP was well-dissolved in the aquadest because, in neutral pH (pH above the value of pKa), it is ionized into polyphosphoric ion ($P_3O_{10}^{5-}$) and natrium ion (Na+). Subsequently, the polyphosphoric ions ($P_3O_{10}^{5-}$) of TPP interact with the cationic group of chitosan ($NH_3^+$) (Lam et al., 2006).

Carbamazepine chitosan nanoparticle formation via the ionic gelation method involved a cross-link reaction between Na-TPP (polyanionic), chitosan (polycationic), and drug (Iswandana et al., 2013). The interaction between carbamazepine (a drug used in this study), chitosan, and Na-TPP forming a nanoparticle, is illustrated in Figure 6. The carbamazepine entrapment between chitosan molecules was due to the interaction between the carbonyl group of carbamazepine and the $NH_3^+$ group of chitosan. Higher electronegativity of atom O than C in the carbonyl group induce the resonance within the C-O covalent bond, which displaces the one pair electron onto atom O to form an intermediate with a negative charge on oxygen atom (C□□-O□□□□□ (Dwiyanti, 2014). Subsequently, the cation amino in chitosan bind with O- atom in the carbamazepine carbonyl group via ionic interaction. The formation of nanoparticles is then completed by inter- and intra-molecular cross-linkage between the chitosan and Na-TPP molecule, which further entrapped carbamazepine inside the particle (Figure 1).

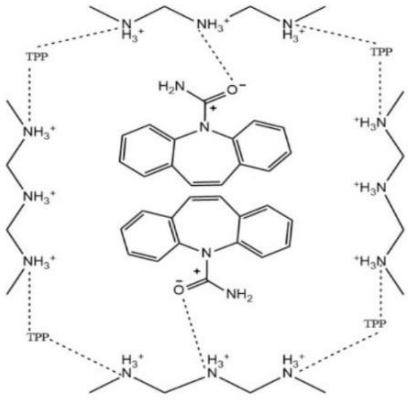


**Figure 1.** Illustration of chemical interactions formed between carbamazepine, chitosan, and TPP

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The preliminary study was conducted to determine the stirring speed and time in preparing carbamazepine chitosan nanoparticles. Based on the results (not published), the best stirring condition was at 350 rpm for 10 minutes, based on the formation of the clear suspension without any hovering particle. According to Apriliyati (Apriliyati et al., 2020), the stirring speed and time affect the particle size and distribution of the nanoparticles. A longer stirring period leads to smaller particle size. However, too long and quick stirring increases the particle size and distribution. A previous study stated that at a higher stirring speed and time, a turbid unclean solution is formed. Therefore, the transmittance percentage value becomes smaller, and the particle size becomes bigger (Abdassah, 2017). According to Fan et al., (2012), the increasing stirring speed accelerates the dispersion of Na-TPP, which affects the shifting power of chitosan and causes the broken interparticle bond-forming aggregation. Therefore, stirring with optimum speed and time makes the formed particle more uniform and equal (Syaputra et al., 2020). Meanwhile, the stirring needs to be maintained to avoid air bubbles within the solution which can inhibit the interaction of the chitosan amino group and polyanion of Na-TPP during the formation process of nanoparticles (Kurniawan, 2012).

Before drying the dispersed particle, a preliminary test based on the measurement of the transmittance percentage was conducted using a UV spectrophotometer to view the solution clarity. A target for good nanoparticle dispersion clarity was the transmittance percentage of over 90% (Huda & Wahyuningsih, 2016), and all formulae met the target. Results of transmittance percentage values of the three formulae were not significantly different ($p>0.05$). These results failed to provide any information because it was on the preliminary test to estimate the size within the nanometer range and could not precisely identify the particle size. Therefore, the particle size was further analyzed using the Particle Size Analyzer (PSA).

The transmittance percentage was similar to the particle size since it was affected by the Brownian Motion, which causes the particles to collide in an irregular direction. Similarly, it is defined as the repulsion motion of inter-particles with the same charge. This Brownian Motion can also maintain the colloid stability against nanoparticles to avoid easy precipitation. Moreover, lesser Brownian Motion occurs due to larger particle sizes that combine to become aggregate and precipitate easily (Abdassah, 2017; Martien et al., 2012). This process causes the transmittance percentage value to become smaller. Visually, the dispersion of nanoparticles appeared transparent and clear (Figure 2), and none of F1, F2, and F3 hovered. The dry powder from freeze-drying also became white, light like cotton (Figure 2), and without odor.


**Figure 2.** Carbamazepine chitosan nanoparticle in the form of dispersion (a), and powder (b)

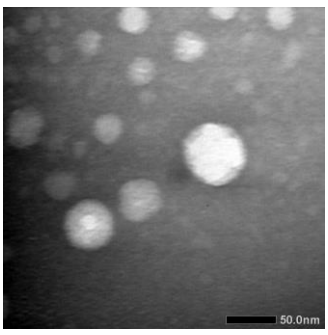


**Figure 3.** TEM image of carbamazepine chitosan nanoparticles at 80000x magnifications

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**Table 2.** Characterization result of carbamazepine chitosan nanoparticles

| Formula (Chitosan: Na-TPP) | Weight ratio Chitosan: Na-TPP (w/w) | Particle size (nm) | Polydispersity index | Zeta potential (mV) | Entrapment efficiency (%) |
|---|---|---|---|---|---|
| F1 (0.2%:0.1%) | 10:1 | 422.4±39.22 | 0.512±0.07 | +7.76±2.03 | 88.00±1.73 |
| F2 (0.2%:0.2%) | 5:1 | 233.5±36.43 | 0.353±0.03 | +17.37±2.67 | 89.00±2.64 |
| F3 (0.3%:0.1%) | 15:1 | 169.8±13.71 | 0.378±0.02 | +28.82±2.44 | 84.30±7.50 |

**Particle size and polydispersity index**

From Table 2, the particle size results of all formulas were categorized as nanoparticles because they fall within the range of 10-1000 nm (Laili et al., 2014). The increased concentration of chitosan often produced the bigger the particle size (Mannuela 2016). However, based on Table 2, the higher concentrations of chitosan and NA-TPP produced a smaller particle size ($p<0.05$). This may be due to when the chitosan concentration was increased, not all chitosan molecules are dissolved to form a linear long chain. Some were still in a coiled state (a smaller and compact form). Therefore, a smaller particle was formed when TPP interacted with the polymer still in the coiled state. In low concentrations of chitosan, such as in F1, the polymer was still in the position of a dilute regime (the linear long-chain state), which causes it to move freely. Therefore, it is difficult for the TPP molecule to interact with chitosan, which leads to higher size (Sreekumar et al., 2018). This finding was supported by the study of Cai (2017), which stated that the size of chitosan nanoparticles not only depends on chitosan concentration but also on the concentration of counterions. If the concentration or weight ratio between the counterions to chitosan was low, crosslinking between polymers is not optimal, which results in a more loose density between two polymers, increasing the size of the particles.

Meanwhile, the higher the counterions to chitosan ratio tends to generate smaller particles due to a higher crosslink density. However, if the concentration of TPP is excessive, this condition can cause aggregation between two chitosan particles resulting in a much bigger size. Therefore, an optimization in the ratio of counterions to chitosan is needed.

The polydispersity index results in Table 2 showed that only F2 and F3 have a value less than 0.5. Therefore, it can be concluded that F2 and F3 formulations resulted in a homogenous particle size because the lower value of PDI signifies a narrower size distribution of the particles. A homogenous size of particles in nanoparticle formation is needed to maintain physical stability by preventing the particle from aggregating (Mardliyati et al., 2012). F3 was the best formula from these two parameters because it has the smallest yet homogenous size of the particles. Thus, it can be easily absorbed in the administration site due to the increase in contact surface area. Smaller particles tend to have a higher solubility and a faster dissolution rate. Oxcarbazepine, an anti-epileptic drug encapsulated into nanoparticles (with the size of 170 nm) was proven to successfully penetrate the Blood-Brain Barrier (BBB) (Lopalco et al., 2015) in the same manner as carbamazepine.

Similarly, the nanoparticle of zaleplon also had a better anti-epileptic effect than free drugs with nanoparticle sizes closer to 200 nm (Haggag et al., 2021). In this study, F3 nanoparticles having a size of 169.8±13.71 nm is the potential to be used as a vehicle to transport carbamazepine and other epileptic drugs to the target site via oral administration. However, the carbamazepine nanoparticle should be further tested in vitro and in vivo to determine its performance.

**Zeta potential**

Zeta potential is a parameter that can be used to predict the nanoparticle stability-based resultant in inter-particle charge, which affects the interparticle repulsion (Mannuela, 2016). The zeta potential is an electrostatic potential that exists on the nanoparticle surface (Mannuela, 2016). Based on the result shown in Table 2, a higher concentration of chitosan concentration increased the zeta potential value to approximately +30 mV ($p<0.05$. A previous study stated that nanoparticle which has zeta potential value closer to or higher than ±30 mV is stable, thus preventing further aggregation and flocculation (Mohanraj & Chen, 2006). This is because the repulsion force is bigger than the attraction force, which leads to an increase in its physical stability (Fitri et al., 2019). Since F3 has the highest value of zeta potential, therefore, it was assumed that F3 has the best physical stability.

Moreover, the value of zeta potential was affected by the chitosan concentration. Zeta potential could have a positive or negative value based on the net charge of the polymer used in the formation of the nanoparticles.

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In this study, all the formulas resulting a positive zeta potential due to the positive charge of chitosan (Arya et al., 2015). A positive charge of zeta potential is beneficial in the drug's transport through the membrane because it facilitates the interaction with a negative charge of the cell membrane and increases its contact length (Bernkop-Schnürch, 2005). The average size and PDI of the resulting nanoparticles also influenced the value of zeta potential. As seen in Table 2, formulas with lower nanoparticles have higher zeta potential. This was due to the distance between two particles being far enough to prevent aggregation, as smaller particles dispersed better in the medium. On the contrary, the bigger particle will have a narrow inter-particle distance, which induces attraction and low zeta potential value (Bernkop-Schnürch, 2005).

**Entrapment efficiency**

The entrapment efficiency describes the percentage of carbamazepine entrapped into a nanoparticle. According to Mannuela (2016), an ideal nanoparticle should have an entrapment efficiency value ≥60%. From the result shown in Table 2, all formulas have an entrapment efficiency of ≥60%. All three formulas have entrapment efficiency values between 84-89%, but it is not significantly different in statistics ($p>0.05$).

**Selection of best formula**

Table 2 showed that among the three formulas, F3 produced the smallest particle size of 169.8±13.71 nm, with a good polydispersity index of 0.378±0.02, the highest value of zeta potential (28.80±2.44 mV), and good entrapment efficiency (84.3±7.50%). Therefore, this showed the weight ratio between chitosan and NA-TPP of 15:1 was the optimal condition to produce nanoparticles of carbamazepine in this study. F3 was further subjected to morphology analysis using TEM.

**Morphology of carbamazepine chitosan nanoparticles**

The analysis result of the TEM morphology of carbamazepine chitosan (F3) is shown in Figure 3. Based on the result, the particle resulting from the F3 formula have spherical-shaped with varied sizes. Most of the particles have sizes lower than 50 nm. A spherical-shaped particle could easily penetrate the cellular membrane, t. Therefore, its penetration was better than that of non-spherical (Martien et al., 2012; Wissing et al., 2004).

Contrary to this result, analysis of the particle using another instrument (Malvern zeta sizer) indicate a formation of the bigger particle but the monodisperse distribution of the particle (PDI <0.5). This was due to a difference in the size determination method in the two instruments. The particle size from a zetasizer was calculated from the hydrodynamic radius of the particle and cumulant average size of all of the particle population, which was also affected by the shape of the particle. However, TEM measurement only shows an image from a tiny portion of the sample which cannot reflect all the particles in the sample. Another reason is that PSA measured the distribution of the dispersion and its reduction. Therefore, its hydrodynamic coverage became broader and produced a bigger particle size, while TEM showed the actual size of the nanoparticle without weighing on the influence of the medium. Therefore a much smaller size is produced (Sulistiawaty et al., 2015).

## CONCLUSION

Based on this study, the increase in chitosan concentration affected the decrease in the particle size ($p<0.05$) and the increase in the value of zeta potential (<0.05), but did not influence the entrapment efficiency value ($p>0.05$). Therefore, the best formula is from the ratio of chitosan and NA-TPP concentrations of 0.3%:0.1% b/v (F3), with the particle size of 169.8±13.71 nm, polydispersity index of 0.353±0.03, a zeta potential of +28.82±2.44 mV, entrapment efficiency of 89±2.64%, and spherical particle morphology.

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## AUTHOR CONTRIBUTIONS

Conceptualization, C.A.E.; Software, A.N.Z.; Methodology, C.A.E.; Validation, C.A.E., A.N.Z.; Formal Analysis, C.A.E., A.N.Z.; Investigation, C.A.E., A.N.Z.; Resources, C.A.E., A.N.Z.; Data Curation, C.A.E., A.N.Z.; Writing - Original Draft, C.A.E., A.N.Z.; Writing - Review & Editing, C.A.E., A.N.Z., W.P., D.A.A.; Visualization, C.A.E., A.N.Z.; Supervision, C.A.E., A.N.Z.; Project Administration, C.A.E., A.N.Z.; Funding Acquisition, C.A.E.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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# Analysis of Factors Affecting the Stress Level and Self-Medication Practice in Primary Dysmenorrhea in Adolescents during COVID-19 Pandemic

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***Abstract***

***Background****: During the novel coronavirus (COVID-19) pandemic, reports of adolescent pain and worsening of the menstrual cycle increased with increasing stress levels.* ***Objective:*** *Analysis of determinant factors that influence the ability to self-medication for menstrual pain, including socio-demography and medical history, to determine the effect of stress levels on the menstrual cycle of adolescent women during the COVID-19 pandemic.* ***Methods:*** *The method is descriptive observational, through a cross-sectional study on adolescent females in Indonesia was conducted in October-November 2020. Data collection through a simple survey method using a self-medication online questionnaire survey and the Kessler psychological distress scale (KPDS) by consecutive sampling technique. About 258 women agreed to participate in the study. The results are presented in the form of frequency and percentage (%), and the determinants are analyzed using $\chi^2$-test and binary logistic regression models.* ***Result:*** *About 78.3% of respondents had stress during the pandemic COVID-19 with primary dysmenorrhea (70.2%), and 58.9% of respondents had low self-medication for managing primary dysmenorrhea. The predictors of self-medication understanding were the mother's education level and history of menstrual pain (p-value = 0.045; 0.005). In addition, respondents' stress levels during the COVID-19 pandemic affected their menstrual cycles (OR=5.110; 95% CI: 1.767–14.782, p-value=0.003).* ***Conclusion:*** *The role of pharmacists in the community and mothers as pioneers of family health is vital in increasing the understanding and implementation of the practice of self-medication for primary dysmenorrhea. This role needs to manage the stress of adolescent women. It can affect the reduction in the menstrual cycle, especially during the COVID-19 pandemic.*

***Keywords****: COVID-19 pandemic, dysmenorrhea, menstrual cycle, pandemic stress, self-medication*

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## INTRODUCTION

In Indonesia, the Novel Coronavirus-19 (COVID-19) infection has occurred since March 2, 2020, and is currently regarded as a global threat (Nugraha et al., 2020). This condition leaves an impact on many things, one of which is secondary health problems in women. Stress as a result of the pandemic causes various issues such as decreased movement in activities, online learning, layoffs, family financial problems, and isolation due to the COVID-19 infection. These become challenges for millions of women of childbearing age. Pandemic stress can disrupt physiological processes related to fertility, such as the menstrual cycle and genital tract health (Aolymat, 2021). The pandemic worsens the quality of social relationships, causes the lack of social support and knowledge related to the health impacts of psychological conditions such as loneliness, and worsens mental and physical health problems (Cohut, 2021). Menstruation is a normal physiological state important in the life cycle and is an integral part of women's health (Thiyagarajan et al., 2020). It indicates the reproductive system's maturity, which can affect most women's physical, mental, and social health (Critchley et al., 2020).

The problem that commonly occurs in almost 50%-80% of women is excessive menstrual pain or dysmenorrhea. The condition of dysmenorrhea is different from that of premenstrual syndrome (PMS), and it can be categorized into two types: primary and secondary (Arafa et al., 2018). Secondary dysmenorrhea is caused by an identifiable pathological condition such as intrauterine devices, endometriosis, inflammation of the pelvis, or the presence of ovarian cysts. Meanwhile, primary dysmenorrhea is associated with no pelvic or hormonal health problems, and this type is most commonly found in adolescent girls (Miller,1988). The occurrence of dysmenorrhea can be followed by menstrual bleeding and abnormal menstrual cycles. This condition can be associated with pandemic stress which affects menstrual patterns (Demir et al., 2021; Phelan et al., 2021; Ozimek et al., 2022). The adrenal gland will release the cortisol hormone to control the resulting stress response. It will suppress the production of reproductive hormones from the ovaries (Thau et al., 2021). Menstrual pattern changes include abnormal menstrual periods, heavy menstrual flow, abnormal cycles, absence of menstruation for several months, and increased pain during menstruation (Ansong et al., 2019).

These studies linked stress levels during COVID-19 to the menstrual cycle. Research conducted by Ozimek (2022) shows the implications of the COVID-19 pandemic on the menstrual cycle. This happened due to the low mental health outcome in women, which interferes with res the women's reproductive function and increases stress conditions (Ozimek et al., 2022). This research seems consistent with that of Demir et al. (2021), who argue that during the COVID-19 pandemic, there was an increase in anxiety and stress levels due to the COVID-19 outbreak, which affected the menstrual cycle characteristics. The other influencing factors are the variance of late sleeping time (Dhawan & Hernole, 2020), lifestyle changes, and increased stress during lockdown that lead to the abnormal menstrual cycle (Bruinvels et al., 2021; Takmaz et al., 2021). Research by Nguyen et al. (2021) shows that the COVID-19 pandemic does not cause changes in women's menstrual cycle and ovulation. However, some women experience abnormalities during the pandemic. This condition is influenced by education level and occupation, where the respondents in this study were mostly well educated (high school – bachelor's degree), had parents (mother) aged 30 years and had a job (not a housewife) (Nguyen et al., 2021). Thus, age, education, maturity, and work will affect women's psychological condition during the COVID-19 pandemic. In addition, research by Nareswari et al. (2021) showed no significant relationship between stress levels during the Covid-19 pandemic and changes in the menstrual cycle (Nareswari et al., 2021).

Menstruation problems significantly negatively impact the socioeconomic burden, one of which is health services (United Nations, 2020). This is due to the fear of contracting COVID-19, the high cost of therapy, and inadequate literacy about self-medication, all of which will worsen the health situation. Self-medication through the prudent, autonomous, and reasonable use of herbal and chemical treatments and the appropriate selection of medicines are feasible solutions to these problems. Self-medication is an individual effort to overcome the disease symptoms without consulting a doctor or asking for a doctor's prescription for treatment (Bennadi, 2014). The rational use of medicines is needed by every woman when dealing with pain or discomfort due to menstruation. Before the COVID-19 outbreak, the prevalence of acute-moderate menstrual pain in women diagnosed with primary dysmenorrhea occurred in approximately three-quarters of all young women under 25 worldwide (Armour, Parry, Manohar,

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et al., 2019). Menstrual pain can be accompanied by cramps, back and thigh pain, headaches, diarrhoea, nausea, and vomiting (Hennegan et al., 2019). This condition will encourage women to find a medicine that provides quick healing regardless of the side effects.

Non-pharmacological strategies such as consuming traditional herbs and controlling physical or psychological activities through meditation and yoga can be used as an alternative to self-medication before deciding to take analgesics as pain relievers (Armour, Parry, Al-Dabbas, et al., 2019). However, these strategies are still rarely applied by adolescent females (De Sanctis et al., 2020; Parra-Fernández et al., 2020). Most prefer to consume instant drinks claimed to have pain relief or even take over-the-counter (OTC) analgesics, such as ibuprofen and or acetaminophen, regardless of the recommended dose and side effects (Matyas et al., 2015). The rational behaviour of self-medication is also determined by education, health workers, parents' role and social support. This collaboration will reduce the barriers that cause inaccuracies in drug selection and factors that trigger menstrual problems, such as good stress management, especially during this pandemic (Rifati & Sudiarti, 2020).

The low understanding of adolescent women toward self-medication as an effective self-care strategy will impact poor pain management. As a result, this study aims to identify the prevalence and factors that influence pandemic stress and its relationship to the changes in menstrual patterns. This study serves as the basis for promotive and preventive measures conducted by health workers, one of which is a pharmacist because it is part of the initial screening and assessment factors that can affect menstrual patterns and health problems. A possible effort that can be carried out in pharmaceutical education for adolescent females based on the causative factors so that the quality of life related to women's health during the COVID-19 pandemic will be well controlled.

## METHODS

### Research Design

A cross-sectional online survey of adolescent females aged 15 to 21 in Indonesia was conducted in October-November 2020 using a consecutive sampling technique. The inclusion criteria in this study were adolescent females aged 15-21 years, menstruated, with no comorbidities such as cardiovascular disease, haematology, acute kidney failure, end-stage renal disease, and cancer. The exclusion criteria were those who did not answer all questions in the online survey. The online questionnaire had undergone expert judgment evaluation and had been given informed consent.

The total population of adolescent females in Indonesia in 2019 was 10.816.900 (Badan Pusat Statistik, 2020). The sample size was calculated based on the population frequency to maintain statistical power for the statistical tests used in the data analysis. The number of samples was determined based on the overall population with a 95% confidence interval, obtaining a minimum sample size of 255 with the OpenEpi Version 3 application, an open-source calculator. The population size (for the finite population correction factor) (N) was 10.816.900, and the percentage (%) frequency of the hypothesized outcome factor in the population was (p): 21% +/- 5, with the confidence interval as +/- 5% of 100 (absolute +/- %) (d), and the design effect (for the DEFF cluster survey) was one so that a minimum sample size of 255 was obtained with a 95% confidence interval. Until the end of the study, 258 participants who258 participants were willing to participate in the survey through online informed consent.

### Data collection method

The data was collected through online surveys from October-November 2020. Privacy and anonymity of survey responses were ensured for all participants. Invitations were announced on the social media used by the researchers to be spread based on the study's inclusion criteria. Participants were also able to invite other friends to participate in the study. The research survey was completed using a Google form linked to the participant's email to prevent duplication. Approval and search instructions are available on the Google Form's home page.

The instruments employed in this study were a questionnaire on sociodemographic characteristics (age, gender, level of education, parents' occupation, location, and parents' income), a health assessment questionnaire, a self-medication questionnaire in overcoming menstrual pain pharmacologically and non-pharmacologically, and Kessler Psychological Distress Scale (KPDS) to determine the stress level. This questionnaire had undergone validity and reliability measurement (Table S1). Based on Cronbach's Alpha measurement, the KPDS questionnaire has a reliability value of $\alpha > 0.8$ (Tran et al., 2019). A questionnaire on the self-medication practice for primary dysmenorrhea

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(Table S2) had passed the expert judgment by doctors, pharmacists, and psychologists. Furthermore, validation and reliability tests were conducted on 30 junior and senior high school students who were not involved as participants in the study. The Cronbach's Alpha analysis results found that all items were declared reliable with $\alpha > 0.060$. Data obtained directly from the participants, including the sociodemographic characteristics, COVID-19's impact on the family economy, health assessment (history of illness, history of dysmenorrhea, menstrual pain based on a visualizable scale with online emoticon, duration, and menstrual cycle), questionnaire of self-medication to treat menstrual pain both pharmacologically and non-pharmacologically, and a stress level questionnaire based on KPDS during this COVID-19 pandemic. The procedure employed in this study is presented in Figure 1.

**Statistical analysis**

The respondent's socio-democratic characteristics and basic health assessment were analyzed using descriptive statistics and were presented in tabular form with variable frequency and percentage (%). Chi-square test as a univariate analysis was used to determine the effect between the independent and dependent variables (stress level, menstrual cycle, and the self-medication ability for menstrual pain), and a p-value $\leqslant 0.050$ was obtained, indicating that the result was statistically significant. In addition, it was also utilized to detect the candidate's variables for the binary logistic regression test on self-medication (p-value $\leqslant 0.250$). For multivariate analysis, binary logistic regression and categorical data on an ordinal/nominal scale were used. It can assess the determining variables of self-medication for menstrual discomfort. If the p-value is less than 0.050, it is considered statistically significant.

**Research ethics**

Ethical approval for this study was obtained from the Ethics Committee of Alma Ata University (approval number KE/AA/VIII/10251/EC/2020). The flowchart describing the research procedure can be seen in Figure 1.

## RESULTS AND DISCUSSION

### Assessment of adolescent women's socio-demography and health during the COVID-19 pandemic

COVID-19 has an impact on adolescents' health quality and stress levels (Ozimek et al., 2022). For some women, this condition affects their menstrual cycle. Prolonged periods of stress in women can suppress the HPA axis, which may affect follicle-stimulating and luteinizing hormones (FSH and LH). This condition affects females' reproductive health during the pandemic (Sharp et al., 2021; Edelman et al., 2022). In addition to hormonal factors, sociodemographic characteristics such as age, parental occupations, educational levels, and income can affect stressful conditions during the COVID-19 pandemic. Research by Caycho-Rodríguez et al. (2021) shows that the COVID-19 pandemic significantly and positively predicted anxiety and depressive symptoms influenced by sociodemographic factors (Caycho-Rodríguez et al., 2021). Therefore, this study descriptively analyzed the prevalence and relationship between stress levels and menstrual cycles according to sociodemographic characteristics.

The assessment results of the sociodemographic characteristics and health history in this study are shown in Table 1. Most respondents had a mean age ($\Delta \pm SD$) of 15.53±0.63 and belonged to the young adult category (17.14±0.43). The fathers' employment status in this study was divided into permanent and temporary workers. The difference lies in the income generated each month. As for the mothers' social status, almost half of them were homemakers. The respondents' parents' education level was mostly higher respondents parents' education level was mostly higher in diplomas and bachelor's degrees. Parents' education and work are crucial for children's health, especially in the transition period to adapt to new habits during the COVID-19 pandemic. Based on the occupation and educational background, 45% of the total sample was affected by economic recession during this pandemic. This study shows that not all sociodemographic characteristics and health history assessments affect the respondents' stress analysis and menstrual cycles during the COVID-19 pandemic (Table 1).

Genital and reproductive health in adolescence is regarded as necessary. Sociodemographic characteristics and health history can influence both. They can also affect adolescent female hormones psychologically and/or physically. The bivariate analysis showed that the respondents' stress levels influenced menstrual duration. The pandemic stress may bring about this condition in women during adolescence. This stress level was experienced by 78.30% of respondents based on the KPDS questionnaire result. Although there is no high risk of death in teenagers compared to adults and geriatrics, they risk experiencing a high-stress level. This condition corroborates with the

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result obtained from this study, where most of the respondents experienced pandemic stress. Changes in their daily activities during the pandemic leave an impact both psychologically and physiologically (Fitzsimmons, 2019; Thibaut & van Wijngaarden-Cremers, 2020). These results are in line with the first non-representative research from China, India, Brazil, the United States, Spain, Italy, and Germany, showing that teenagers during this pandemic tend to have negative mental health (Ravens-Sieberer et al., 2021).

This condition affects the respondents' menstrual duration with an odds ratio (OR) value of 95% CI 1,250 (1.122-1.393). This means that people with pandemic stress have a 1.25-times risk of experiencing irregular menstrual periods compared to those without pandemic stress. Furthermore, problems related to menstrual duration are linked to changes in the menstrual cycle (Rafique & Al-Sheikh, 2018; Edelman et al., 2022). Respondents with irregular menstrual periods had an average menstrual duration of 28.00±4.91 days, while the average cycle and duration for normal menstruation was 26.00±4.51 days with an OR value of 95% CI 3.071 (1,570-6,010). These results indicate that individuals with irregular menstrual duration have a 3.07 times greater risk of experiencing problems related to the menstrual cycle than those with normal menstrual duration. This shows that stress levels may affect menstrual cycles during the COVID-19 pandemic, with [OR] = 5.110; 95% CI 1.767–14.782, p-value=0.003).

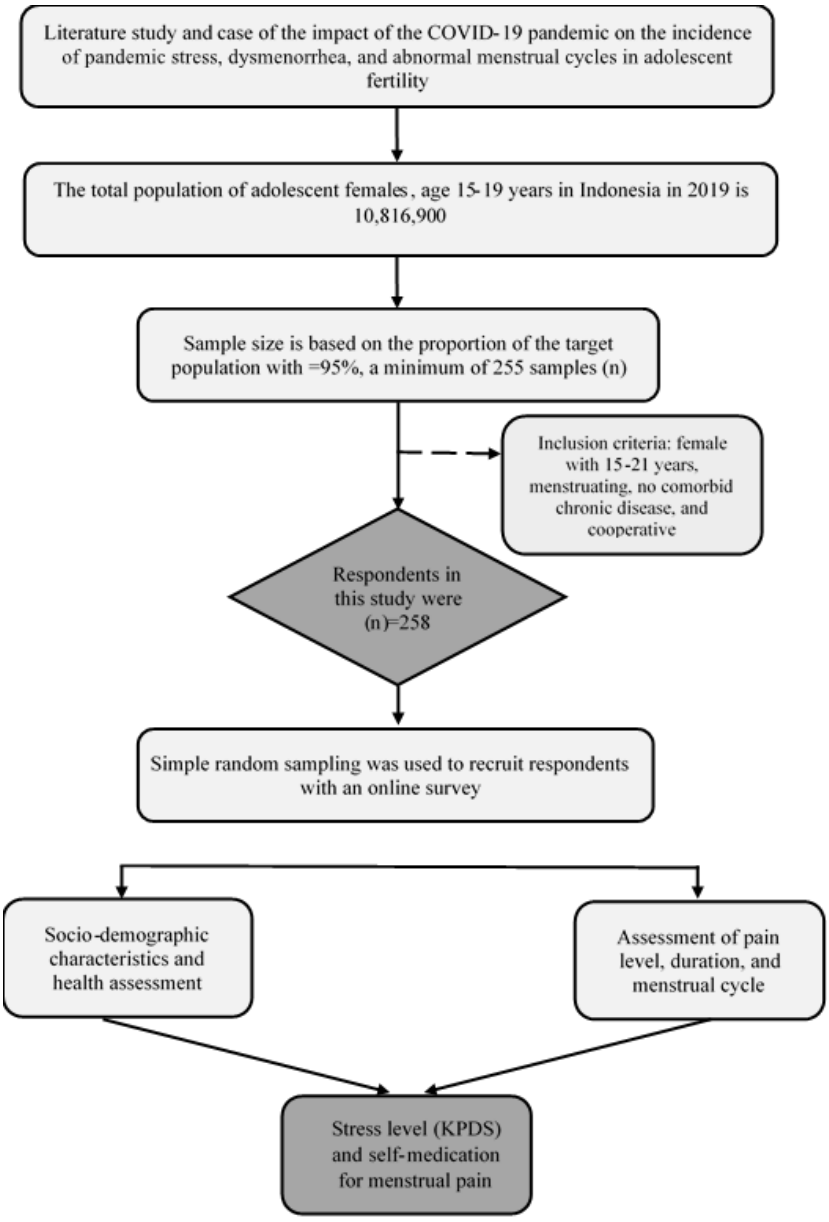


**Figure 1**. Research procedure

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**Table 1.** Prevalence of stress levels and menstrual cycle based on sociodemographic characteristics and health of respondents

| Socio-demographic & health characteristics | Total (n=258) n (%) | Stress analysis - based on Kessler (n=258) | | $\chi^2$ | *p-value* | Menstrual Cycle (n=258) | | $\chi^2$ | *p-value* |
|---|---|---|---|---|---|---|---|---|---|
| | | No n (%) | Yes n (%) | | | Normal n (%) | Abnormal n (%) | | |
| **Age (years)** | | | | | | | | | |
| 15-17 | 173(67.1) | 35(62.5) | 138(68.3) | 0.672 | 0.413 | 132(67.0) | 41(67.2) | 0.001 | 0.975 |
| 18-21 | 85(32.9) | 21(18.3) | 64(66.6) | | | 65(33.0) | 20(32.8) | | |
| **Father's job description** | | | | | | | | | |
| Permanent | 132(51.2) | 27(48.2) | 105(52.0) | 0.249 | 0.618 | 98(49.7) | 34(55.7) | 0.669 | 0.413 |
| Temporary | 126(48.8) | 29(51.8) | 97(48.0) | | | 99(50.3) | 27(44.3) | | |
| **Mother's job description** | | | | | | | | | |
| Workers' | 127(49.2) | 33(58.9) | 94(46.5) | 2.695 | 0.101 | 100(50.8) | 27(44.3) | 0.787 | 0.375 |
| Housewives' | 131(50.8) | 23(41.1) | 108(53.5) | | | 97(49.2) | 34(55.7) | | |
| **Father's education** | | | | | | | | | |
| Primary and secondary | 18(7.0) | 4(7.1) | 14(6.9) | 0.003 | 0.959 | 13(6.6) | 5(8.2) | 0.183 | 0.669 |
| Higher | 240(93.0) | 52(92.9) | 188(93.1) | | | 184(93.4) | 56(91.8) | | |
| **Mother's education** | | | | | | | | | |
| Basic | 53(20.5) | 12(21.4) | 41(20.3) | 0.034 | 0.853 | 158(80.2) | 47(77.0) | 0.284 | 0.594 |
| Higher | 205(79.5) | 44(78.6) | 161(79.7) | | | 39(19.8) | 14(23.0) | | |
| **Economic impact** | | | | | | | | | |
| Yes | 116(45.0) | 30(53.6) | 86(42.6) | 2.143 | 0.143 | 89(45.2) | 27(44.3) | 0.016 | 0.900 |
| No | 142(55.0) | 26(46.4) | 116(57.4) | | | 108(54.8) | 34(55.7) | | |
| **Menstruation duration** | | | | | | | | | |
| Normal | 211(81.8) | 53(94.6) | 158(78.2) | 7.939 | 0.005* | 170(86.3) | 41(67.2) | 11.383 | 0.001* |
| Abnormal | 47(18.2) | 3(5.4) | 44(21.8) | | | 27(13.7) | 20(32.8) | | |
| **Menarche** | | | | | | | | | |
| ⩽13 year | 208(80.6) | 7(43.8) | 54(87.1) | 14.020 | 0.001* | 44(77.2) | 17(81.0) | 0.127 | 0.721 |
| >13 year | 50(19.4) | 9(56.3) | 8(12.9) | | | 13(22.8) | 4(19.0) | | |

n= number of samples in the study; *$\chi^2$=value Pearson Chi-Square; *p-value<0.050 a significant relationship between variables in demographics and health with stress and or the menstrual cycle*

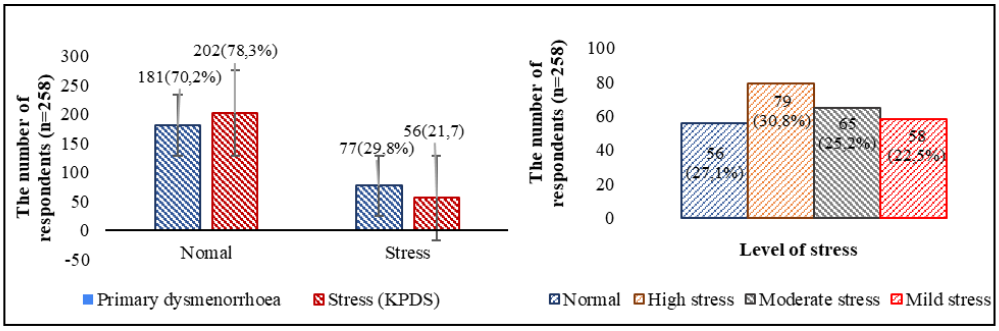


**Figure 2.** Primary dysmenorrhea prevalence and stress levels based on Kessler (KPDS questionnaire)

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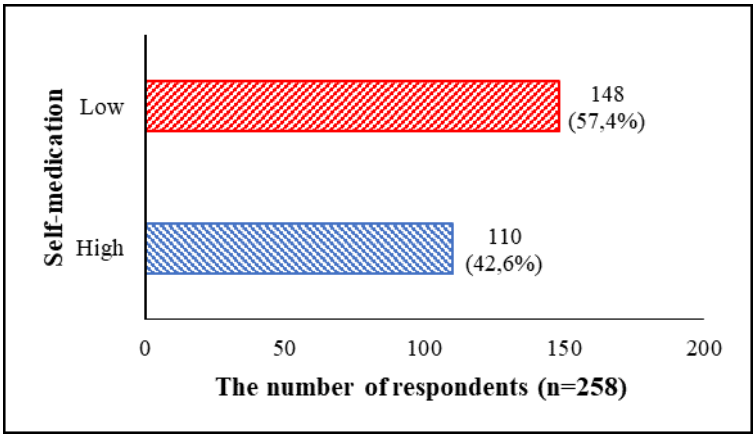


**Figure 3.** Primary dysmenorrhea self-medication ability during the COVID-19 pandemic

Problems in the menstrual pattern are associated with the level of stress during the COVID-19 pandemic (Edelman et al., 2022). Most respondents (80.6%) had a regular menstrual duration during the pandemic with menarche that occurs at the age of 13. The age of menarche suggests the early age of women experiencing menstruation. This study relates to the pandemic stress experienced by the participants. Statistically, it shows the relationship between the two variables with OR 95%CI power value of 1.881 (1.127-3.140). Adolescent females who experience menarche at a relatively young age have e 1.88 times greater risk of having poor stress management characterized by an increased stress level compared to that of general age. Commonly, women experience menarche at 10-16 years old, and some start at 14-17 years old (middle adolescence).

**The prevalence of primary dysmenorrhea and stress levels during the COVID-19 pandemic**

From the results of this study, the prevalence of primary dysmenorrhea is shown in Figure 2. It reveals that based on the measurement of stress levels using the KPDS questionnaire, primary dysmenorrhea occurred in respondents who experienced stress. A total of 143 (55.40%) respondents experienced primary dysmenorrhea with increased stress levels during the COVID-19 pandemic.

Problems in the menstrual cycle can be caused by psychological stress. The pandemic stress that occurred in most respondents affected their menstrual patterns, such as the menstrual cycle and primary dysmenorrhea. The indirect effects came from stress, anxiety, malnutrition, and physical activity, which affect reproductive health and menstrual patterns (McNamara et al., 2020). The menstrual cycle is controlled by the hypothalamus-pituitary-ovarian complex's brain centre, which has a positive and negative feedback mechanism. This complex is influenced by the stress conditions experienced by the respondents.

In addition, adequate energy plays a role in the availability of gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus. It will alter the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. It reduces estrogen and progesterone levels (Mikhael et al., 2019). Some hormonal disorders can influence the menstrual cycle, duration, and primary dysmenorrhea. Menstrual cycles last 25-30 days in most women (Ferin, 2008). In this study, 61 respondents (23.60%) had menstrual cycles over 30 days. Since the outbreak of the COVID-19 pandemic, menstrual changes have been shown to occur in most women, including duration, frequency, regularity of the cycle, the volume of bleeding, and dysmenorrhea (Yamakoshi, 2020; Aolymat et al., 2022). It is associated with COVID-19 or factors related to psychological stress and changes in healthy behaviours (Sharp et al., 2021b). The level of anxiety and psychological stress may affect a woman's menstrual cycle (Phelan et al., 2021; Jawad et al., 2021). Specific stressors that affect stressful conditions for adolescents during the COVID-19 pandemic are difficulties in accessing health services, changes in the family economy caused by the pandemic, distance learning and parental work (Al Dhaheri et al., 2021). In addition, stress not only occurs due to the pandemic but also due to parenting patterns, parents' occupation and economy, relationship status, nutritional status, and environmental conditions (Bae et al., 2018; Haeriyah et al., 2019; Yilmaz et al., 2021).

Stress level, menorrhagia, and menstrual pain are related to each other (Yamamoto et al., 2009), while the menarche and premenstrual symptoms are significant determinants of primary dysmenorrhea (p-value=0.012)

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(Singh et al., 2015). These results are in line with this study. Menstrual cramps experienced by the respondents underwent an increase in pandemic pressure. Research conducted by Grandi et al. (2012) at the University of Modena and Reggio Emilia in April 2010 found that 55.2% of 500 women would consider the need for self-medication to treat the cause of menstrual pain (p-value<0.001) (Grandi et al., 2012). Although menstrual history becomes the determinant that affects the ability to conduct self-medication practice for menstrual pain, the participant's proper self-medication is generally low. Chen et al. (2019) stated that 34.8% of 2555 women knew how to self-medicate, 15.6% of whom used modern medicine with synthetic drugs, and only 8.60% were able to self-medicate for menstrual pain through the use of traditional herbs. Individuals with greater pain severity were more likely to attempt self-medication (OR = 7.01; 95% CI 4.50-10.91), used complementary therapies (OR = 2.64; 95% CI 1.70–4.10) ,and sought advice from a health professional (OR = 5.93; 95% CI 3.80 to 9.24) (Chen et al., 2019).

**The prevalence of understanding primary dysmenorrhea and self-medication during the COVID-19 pandemic**

The prevalence of adolescent females related to the understanding of self-medication ability by using over-the-counter and non-pharmacological drugs using herbal plants for treating dysmenorrhea is relatively low (Figure 3). Respondents' self-medication efforts need to collaborate with pharmacists, parents, social environment, and other health professionals who provide education on rational self-medication. In this case, individuals will take responsibility for their health and well-being, including maintaining their physical and mental health (Esfandiari et al., 2018; Rutter, 2015).

A cut-off point was used to determine, categorize or continuously dichotomize the ability level to self-medicate primary dysmenorrhea (Table S2). The cut-off point was based on the distribution of the total score of the questionnaire. The scores' distribution based on the data's normality was analyzed to define two cut-off points (Oliveira et al., 2015). The Kolmogorov-Smirnov test was used to analyze the normality of the data, and the result indicates that the data were not normally distributed. Therefore, two cut-off points were made based on the median. The median value of the questionnaire score (Table S2) was 75. If the score $\geqslant$ 75, it means that the level of knowledge is high, and if the score is < 75, then the level of self-medication understanding is low. The result showed that most of the respondents (57.4%) had a relatively low level of self-medication (Figure 3). This prevalence mainly occurred in middle adolescence, with as many as 104 (60.1%) respondents. While in young adulthood, there were as many as 44 (51.8%) respondents with a low level of ability and 41 (48.2%) respondents with good skills in self-medication for primary dysmenorrhea.

In addition to the self-medication ability to treat menstrual pain caused by primary dysmenorrhea, this study showed that most respondents (57.4%) used non-steroidal anti-inflammatory drugs (NSAIDs) such as mefenamic acid and ibuprofen. Furthermore, the non-pharmacological therapy applied was relaxation with aromatherapy oil and massage to relieve dysmenorrhea pain. The low level of self-medication requires education, especially by pharmacists. However, parents also play a pivotal role. Mothers are pioneers in family health (Parra-Fernández et al., 2020). This is shown by the correlation between a mother's education and the respondent's self-medication ability in this study.

**Determinants Factors of Self-Medication to Treat Menstrual Pain during the COVID-19 Pandemic**

This research examines the correlation between all variables (Table 3), including sociodemographic and health assessment results on the respondent's self-medication practice in primary dysmenorrhea. The main influencing factors were analyzed, and the sociodemographic characteristics and health history of the respondents or their parents were found (Table 3). Based on the correlation analysis, several variables had a moderate to weak correlation with understanding self-medication. An analysis of multiple ordinal logistic models and backward conditional elimination techniques were used to figure out those determining factors.

The binary logistic regression was employed to examine whether physical activity, medical history, and sociodemographic characteristics helped to explain self-medication to treat menstrual pain in 258 samples. The finding is presented in Table 3. It also describes the coefficients, Wald test values, p-values, odds ratios, and 95% confidence intervals for odds ratios. When all explanatory variables were treated as continuous variables, such as physical activity, medical history, and sociodemographic characteristics, it was discovered that two of them were statistically significant at the 0.05 level of significance (Table 3). This study aims to evaluate the null hypothesis of the logistic regression model of physical activity, medical history, and

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sociodemographic characteristics, as well as to evaluate the alternate hypothesis of the logistic regression model with all explanatory factors using the Likelihood Ratio (LR) test. If $-\mathbf{2}\ \mathbf{log}(\boldsymbol{\Lambda}) > \boldsymbol{\chi q}, \mathbf{1}-\boldsymbol{\alpha}\ \mathbf{2}$ or p-value < $\boldsymbol{\alpha}$, the null hypothesis is rejected. The LR statistic value in this study was 348,464 and p=0,014 (p0,050), indicating that physical activity, medical history, and sociodemographic factors included in the model are important. As a result, the model included all explanatory variables. The Wald test was used to compare two hypotheses to determine individual regression slope coefficients (H0: $\boldsymbol{\beta r} = \mathbf{0}$vs H1: $\boldsymbol{\beta r} \neq \mathbf{0}$) (Astari & Kismiantini, 2019). The Wald values were obtained by dividing the slope coefficients by their standard error (S.E not shown). If the null hypothesis is true, the Wald value for a large sample has an approximate standard normal distribution. Meanwhile, the null hypothesis is rejected if the Wald value is greater than the critical standard normal value or if the p-value is less than the significance threshold. For example, Table 3 shows that the coefficient for a mother's education and family history of dysmenorrhea are 0.045 and 0.005, and the Wald values 4. are 025 and 8.006, respectively. Given the other explanatory factors in the model, the relevant p-value for this test is 0.050. There is substantial evidence that the mother's education and family history of dysmenorrhea is crucial to be included in the model. These findings corroborate with the study conducted by Lee et al. (2017), who argues that there are factors influencing the irrational use of drugs among adolescents, one of which is to overcome menstrual pain. Irrational self-medication can potentially cause adverse drug reactions (ADR) and worsen health conditions. In 2016, 6,226 adolescents in Taiwan reported that the most frequently reported self-medication drugs included non-steroidal anti-inflammatory drugs or analgesics, with a prevalence rate of 31.1%. Several studies have found that over-the-counter (OTC) use is linked to adverse drug reactions that result in death. These hazards include the use of high doses, prolonged duration of drug use, drug interactions, polypharmacy, drug misuse, and adverse drug reactions (ADR). The most common effect is moderate to chronic gastrointestinal disorders (Lee et al., 2017).

The effect size on the dependent variable is indicated by the exponential value (EXP (B)) or odds ratio (OR). The mother's education had an OR of 0.531, meaning that mothers who do not complete nine years of compulsory education will have a 0.286-times lower risk of self-medication ability level compared to those with higher education (high school, diploma, bachelor's degree). The value of the beta coefficient (β) was 0.634. This shows a positive relationship between a mother's education and the respondent's ability to self-medicate for primary dysmenorrhea. The higher the mother's educational level, the better the ability of adolescent women to self-medicate. The history of dysmenorrhea had an OR of 2.201, meaning that respondents with a previous history of dysmenorrhea will have a higher level of knowledge or 2.201 times in the ability to self-medicate. The beta coefficient (β) result was 0.789, meaning the beta coefficient (β) was positive. The history of dysmenorrhea has a positive relationship with the understanding of self-medication. Based on the coefficient value, the regression equation model was formed as follows:

$$Linear\ equaton = \frac{\exp(-0.101 + 0.634\ \text{Mother}'\text{s education} + 0.789\ \text{History of dysmenorrhea})}{\exp(-0.101 - 0.634\ \text{Mother}'\text{s education} + 0.789\ \text{History of dysmenorrhea})}$$

**Table 3.** Determinant factors of ability primary dysmenorrhea self-medication in logistic regression analysis (multiple ordinal logistics model) during the COVID-19 pandemic

| Predictor factor | Beta coefficient ($\boldsymbol{\beta}$) | Wald test | *p-value* | *Odds Ratio* (OR) | 95%CI | |
|---|---|---|---|---|---|---|
| | | | | | Minimum | Maximum |
| Father's job description | 0.125 | 0.213 | 0.644 | 1.133 | 0.668 | 1.921 |
| Mother's job description | -0.781 | 1.001 | 0.317 | 1.313 | 0.770 | 2.237 |
| Economic impact | -0.185 | 0.496 | 0.481 | 0.831 | 0.496 | 1.392 |
| Father's education | 0.246 | 0.837 | 0.360 | 1.278 | 0.755 | 2.164 |
| Age of respondents | - 0.400 | 1.998 | 0.157 | 0.671 | 0.385 | 1.167 |
| Mother's education | 0.634 | 4.025 | 0.045* | 0.531 | 0.286 | 0.985 |
| Family history of dysmenorrhea | 0.789 | 8.006 | 0.005* | 2.201 | 1.274 | 3.803 |

*****Significantly partial effect on the level of self-medication ability of menstrual pain with p-value<0.050

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According to the linear equation above, if the respondent's mother has a high educational value of '1' and a history of dysmenorrhea of '1', it is predicted that the respondent can provide education by health professionals and parents have a high sensitivity to the lifestyle of teenagers, especially during the COVID-19 pandemic. Stress management is necessary for both physical and mental health. The results of this study can be used as a basis to support and provide education on behavior modification, lifestyle, and the selection of drugs as a means for conducting self-medication wisely and appropriately.

## CONCLUSION

This study's results indicate that most respondents with primary dysmenorrhea experienced moderate until high stress based on the Kessler Psychological Distress Scale (KPDS) questionnaire that was evaluated during the COVID-19 pandemic. The determinant factors that influence stress level are the age of menarche and the length of menstruation in adolescents. The predictors that influence it are the history of dysmenorrhea and the educational level of the mother's educational level. Most respondents have a poor understanding of managing primary dysmenorrhea by self-medicating.

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## AUTHOR CONTRIBUTIONS

Conceptualization, N.K.; Software, I.N.R.; Methodology, N.K.; Validation, E.D.; Formal Analysis, J.A.H.; Investigation, R.S.O.; Resources, N.K., T.S.; Data Curation, N.K.; Writing - Original Draft, N.K., I.N.R., J.A.H.; Writing - Review & Editing, E.D.; Visualization, E.D.; Supervision, N.K.; Project Administration, V.A.F., R.S.O., T.S., R.N.; Funding Acquisition, N.K., I.N.R, J.A.H.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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# Analysis of Drug Use in Non-Ventilator Covid-19 Patients at Bangil Hospital

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***Abstract***

***Background****: Coronavirus Disease 2019 (Covid-19) is an infectious disease. Common signs and symptoms of Covid-19 infection include acute respiratory distress such as fever, cough and shortness of breath. Analysis of drug use in Non-Ventilator Covid-19 patients needs to be carried out to provide an overview of drug therapy currently being used based on the Covid-19 Management Guidelines Edition 3rd because so far, there has not been a single type of drug that has received marketing authorization for Covid-19 therapy.* ***Objectives****: This study aims to determine patient profiles, therapeutic outcomes, suitability of drug use with Covid-19 Management Guidelines 3rd edition, the quantity of antibiotic use using the DDD/100 patient-days method and antivirals.* ***Methods****: The method used in this study is an observational design study which was analyzed descriptively with retrospective data collection.* ***Result****: Based on the profile of drug use in COVID-19 patients, there are 31 types of drug classes. The most consumed drugs were antibacterial (91%) and analgesics (91%), followed by vitamins (89%) and supplements (85%). Based on the DDD/100 patient-days value, the most widely used antibiotics were Azithromycin.* ***Conclusion****: The patient's profile at the time of admission to the hospital was 44% moderate and 56% severe. The condition of patients at the time of discharge from the hospital with a moderate degree using antivirals and antibiotics were more recovered and returned home with improvements with percentages of 84.1% and 88.64%, respectively.*

***Keywords****: covid-19, non-ventilator patients, antibiotic, antiviral*

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## INTRODUCTION

Infectious disease is a disease that often occurs throughout the world. The cause of this infection is a new type of virus called Severe Acute Respiratory Syndrome Coronavirus-2 or SARS-CoV-2. WHO named the disease Coronavirus Disease 2019 or Covid-19 through consultation and collaboration with the World Organization for Animals (OIE) and the Food and Agriculture Organization of the United Nations (FAO). Common signs and symptoms of Covid-19 infection include acute respiratory distress such as fever, cough, and shortness of breath. The average incubation period is 5-6 days, with the most prolonged incubation period being 14 days. Severe cases of Covid-19 can lead to pneumonia, acute respiratory syndrome, kidney failure, and even death.

Transmission of the Covid-19 virus can occur through respiratory droplets (aerosol), direct contact with contaminated objects and surfaces from sufferers, and both asymptomatic and symptomatic (WHO, 2021). On January 30$^{th}$, 2020, this virus spread rapidly and caused an exponential increase in the number of patients until July 9th, 2020. WHO reported 11,840,226 confirmed cases with 545,481 deaths worldwide (Case Fatality Rate/CFR 4.6%) (WHO, 2021). Based on the condition and severity of Covid-19 cases, they are divided into several categories: asymptomatic, mild, moderate, severe, and critical. Based on an earlier study, from the profile of Covid-19 patients for the period January to April 2021 at RSU Haji Surabaya from 100 patients, the results showed that 61% were Covid-19 patients in the moderate category and 39% were in a severe category. Among patients in moderate category, 90.16% recovered while 9.84% died. From the profile of drug use in COVID-19 patients for the period January to April 2021 at RSU Haji from 100 patients, the results showed 99% of patients used mucolytic drugs by 99%, and the second most used was vitamin class drugs, which was 98%, then followed by the use of antibiotics class of drugs by 97%, and the fourth is antiviral drugs by 96% (Isna, 2021).

Following the Covid-19 therapy guidelines from WHO, Indonesia has recommended supportive therapy for handling Covid-19, such as symptom therapy, supplemental oxygen, antibiotics, fluid therapy, vasopressors, and medical measures (including installing a ventilator) to save the patient's life (Setiadi et al., 2020). Repurposing drugs is the use of drugs that already exist and are not officially indicated for Covid-19 but are used as therapy through an efficient approach. (Singh et al., 2020). According to the guidelines for managing Covid-19 therapy in Indonesia, which refers to the WHO, empirical antibiotics are given as therapy for Covid-19 patients to overcome secondary bacterial infections (Burhan et al., 2020). Data on the use of antibiotics in Covid-19 patients shows that antibiotics rank first in the treatment of Covid-19 with a percentage of 90.83% (Gutiérrez et al., 2020). The most widely used antibiotics are ceftriaxone 69.28%, azithromycin 67.93%, and levofloxacin 15.2%. Other therapies used for Covid-19, among others, are antimalarials by 44.37%, with the use of hydroxychloroquine ten times greater than chloroquine (64.95%: 6.13%), the use of steroids by 44.37%, and the use of the antiviral lopinavir-ritonavir by 42.63% and tocilizumab by 9.37% (Gutierrez et al., 2020). Analysis of drug use in Non-Ventilator Covid-19 patients needs to be carried out to provide an overview of drug therapy currently being used based on the Covid-19 Management Guidelines 3$^{rd}$ Edition because so far, there has not been a single type of drug that has received marketing authorization for Covid-19 therapy. Reminding the high cases of Covid-19 and the efforts of all parties to obtain the best therapeutic data in perfecting the management of Covid-19 therapy, this study aims to determine patient profiles, therapeutic outcomes, suitability of drug use with Covid-19 Management Guidelines 3$^{rd}$ edition, the quantity of antibiotic use using the DDD/100 patient-days method, and the use of antivirals at Bangil Hospital.

## METHOD

This research was an observational research design which used a retrospective descriptive design. The data sources were medical records and records of drug use from the Department of Hospital Pharmacy. The population in this research were Covid-19 patients with moderate or severe categories of hospitalization who received treatment at Bangil Hospital. The sample size was taken using the purposing sampling method, namely 100 COVID-19 patients who met the inclusion and exclusion criteria from all confirmed COVID-19 patients in the isolation room without a ventilator (moderate or severe).

Assessment and evaluation of the quantity of antibiotic use is carried out using the Defined Daily Dose. The calculation of the Defined Daily Dose was as follows:

$$\frac{DDD}{100} Patient - Days: \frac{Medicine\ in\ grams\ used\ by\ all\ patient\ x\ 100}{DDD\ WHO\ standard\ in\ grams\ LOS}$$

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The reliability and accuracy of drug use in percentage units compared with the Covid-19 Management Manual 3rd Edition 2020. This research used ICD-10 and ATC codes to shorten the presentation table by grouping. This research also received an Ethics Certificate from the Bangil Hospital Ethics Number 445.1/026/424.072.01/2021.

## RESULTS AND DISCUSSION

Coronavirus disease has been identified since 2019 (Covid-19) and is associated with the cause of many deaths. It has been reported that the most susceptible to Covid-19 are elderly patients with a 6-fold higher risk. Based on the severity of cases, Covid-19 is divided into 5 levels: asymptomatic, mild, moderate, severe and critical. This shows that the treatment or therapy given must be different for each so as not to worsen the patient's condition (PDPI et al., 2020). In this situation, it is necessary to conduct a drug analysis for non-ventilator Covid-19 patients.

Samples were classified by their severity. Each level of severity was further classified based on age, sex, and length of hospitalization. The sample data showed that most of the moderate Covid-19 patients were 36-45 years old (late adulthood) and 46-55 years old (early elderly), with a percentage of 22%. Moreover, most patients with the severe category were 56-65 years old (elderly), with 17%. Based on the length of hospitalization, the severe category takes longer than moderate severity.

Based on the demographic data, the sample was divided according to age and gender. The result showss that the highest percentage is at 36-45 years (late adulthood). This age group is the most affected by Covid-19 because of the frequent contact with patients. Meanwhile, based on gender, the most affected by Covid-19 are males. This is due to hormonal and immune conditions, and viral receptor enzymes are more commonly found in the male. The enzyme in question is Angiotensin-Converting Enzyme 2 (ACE 2) (Esmaeil et al., 2022).

Based on the demographic data, there were 100 samples. 44 samples (44%) were in the moderate category, and 56 samples (56%) were in a severe category. Moderate Covid-19 patients required hospitalization for 456 days, while patients with severe category of Covid-19 need 797 days for hospitalization. Factors that can affect the length of hospitalization are complications experienced by the patient. Patients with comorbidities may experience complications that prolong hospitalization (Hiroyuki et al., 2021).

The results of the research samples in the moderate patient category indicated that there were 24 samples (24%) of male infected with Covid-19 and this number was more than female who only had 20 samples (20%). The results of the research sample for severe patient category based on gender showed that there were 32 samples of male patients (32%) infected with Covid-19, and this number was more than female patients who only had 24 samples (24%). This is in line with a study entitled Pandemic Covid-19 in a Demographic Perspective which stated that there were 51.5% male Covid-19 patients and only 48.5% female (Laura et al., 2021). Other studies have also found different behavior between males and females in maintaining their health. Females tend to care more about their health than males. In general, this behavior shows that females comply more with health protocols such as wearing masks, washing hands and keeping a distance (Goncalves et al., 2020).

**Table 1.** Severity Profile

| **Variable of Covid-19 Patients** | | | |
|---|---|---|---|
| | | **Moderate** | **Severe** |
| Age | 17 – 25 | 7% | 3% |
| | 26 – 35 | 7% | 5% |
| | 36 – 45 | 12% | 14% |
| | 46 – 55 | 10% | 13% |
| | 56 – 65 | 7% | 17% |
| | 65 – over | 1% | 4% |
| Gender | Male | 24% | 32% |
| | Female | 20% | 24% |
| Length of stay | | 456 days | 797 days |
| Mean | | 10 | 14 |

The sample data of severity affects the condition of patients out of the hospital. The exposure data can be seen in Table 2. The data showed that patients with moderate severity had no death cases, while 19 (33.93%) patients died in severe severity. The profile of the study sample based on the condition of discharge from the hospital was influenced by the severity. Patients with symptoms who were discharged from the hospital in a recovered state were more than those who were discharged in an improved state. None of the Covid-19 patients with moderate symptoms died. More patients with severe symptoms of Covid-19 returned home in a recovered state than in an improvement state. An improvement state means that the patients are still given drugs to support their recovery. Meanwhile,

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patients with severe symptoms died. Thus, it can be concluded that the number of patients with recovered outcomes is greater than the number of deaths (Kim et al., 2020).

This study is in accordance with a study in Korea which stated that the study's total sample was 5621 patients, with a total of 5387 patients recovered and 234 patients died. The study also explained that most Covid-19 patients who died were due to worsening conditions and respiratory failure in the ICU (Luis et al., 2022). The criteria for recovered patients are asymptomatic, mild symptoms, moderate symptoms, or severe/critical symptoms, provided that the patients have met the criteria for the issuance of a warning letter based on the assessment of the doctor at the health facility where it is carried out or by the DPJP. The criteria consist of a thorough clinical study that includes improved radiological and blood examination carried out by the DPJP, stating that the patient is allowed to go home. There is no action/treatment required by the patient, either related to COVID-19 or other health problems experienced by the patient (PDPI et al., 2020).

Complication regarding Covid-19 is mainly associated with pneumonia (Table 3). This can occur because Covid-19 attacks the respiratory tract, where there are many ACE2 receptors, causing the respiratory tract to weaken as Covid-19 takes host (Naoyuki et al., 2022). Inflammation of the lungs can trigger infection and worsen the inflammatory condition (Tsamrotul et al., 2020). Inflammatory conditions of the lungs due to infection can be categorized as pneumonia. Inflammation of the airways can lead to respiratory failure in patients. Respiratory failure can cause the patient's condition to worsen and even cause death. This condition needs to be considered so that Covid-19 patients do not experience a much worse condition (Somers et al., 2020).

**Table 2.** Profile of Research Samples Based on Severity and Outcome Patient

| Severity | Outcome Patient | | | |
|---|---|---|---|---|
| | Healed | Repatriation | Died | Total |
| Moderate Category Covid-19 Patients (44%) | 56.82% | 43.18% | 0% | 100% |
| With antibiotics | 50.00% | 38.64% | 0% | |
| Without antibiotics | 6.81% | 4.55% | 0% | 100% |
| | | | | |
| Appropriate antibiotics | 38.47% | 25.64% | 0% | |
| No appropriate antibiotics | 20.51% | 15.38% | 0% | 100% |
| | | | | |
| With antivirals | 54.55% | 29.55% | 0% | |
| Without antivirals | 2.26% | 13.64% | 0% | 100% |
| | | | | |
| Appropriate antivirals | 43.24% | 32.44% | 0% | |
| No appropriate antivirals | 21.62% | 2.70% | 0% | 100% |
| | | | | |
| Severe Category Covid-19 Patients (56%) | 53.57% | 12.5% | 33.93% | 100% |
| With antibiotics | 48.21% | 10.71% | 33.93% | |
| Without antibiotics | 5.36% | 1.79% | 0% | 100% |
| | | | | |
| Appropriate antibiotics | 38.46% | 7.69% | 29.92% | |
| No appropriate antibiotics | 11.54% | 5.77% | 9.62% | 100% |
| | | | | |
| With antivirals | 46.43% | 12.50% | 30.36% | |
| Without antivirals | 7.14% | 0% | 3.57% | 100% |
| | | | | |
| Appropriate antivirals | 44.00% | 14.00% | 32.00% | 100% |
| No appropriate antivirals | 8.00% | 0% | 2.00% | |

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**Table 3.** Profile of Research Samples Based on Diagnosis of Comorbidities

| ICD-10 Code Grouping | Code | Percentage of Patients (%) | Diagnosis | ICD-10 Code | Percentage (%) |
|---|---|---|---|---|---|
| A00-B99 | A | 47 | Gastroenteritis | A08.4 | 12 |
| | | | Tuberculosis | A15 | 3 |
| | | | Sepsis | A41.9 | 34 |
| | B | 2 | Hepatitis C | B17.9 | 2 |
| D50-D89 | D | 14 | Anemia | D64.9 | 2 |
| | | | Hypercoagulation | D68.69 | 11 |
| | | | Thrombocytopenia | D69.6 | 1 |
| E00-E90 | E | 22 | Diabetes mellitus | E10 | 19 |
| | | | Hypoglycemia | E16 | 1 |
| | | | Dyslipidemia | E78.5 | 3 |
| | | | Hyponatremia | E87.1 | 1 |
| | | | Hyperkalemia | E87.5 | 1 |
| | | | Hypoalbumin | E88.9 | 2 |
| H60-H95 | H | 2 | Vertigo | H81.399 | 2 |
| I00-I99 | I | 11 | Hypertension | I10 | 2 |
| | | | Acute Coronary Syndrome/ACS | I24.9 | 3 |
| | | | Heart Failure / HF | I50.9 | 1 |
| | | | Stroke / CVA | 163.9 | 3 |
| | | | Atherosclerosis | I70.9 | 2 |
| J00-J99 | J | 73 | Pneumonia | J12.82 | 69 |
| | | | Bronchitis | J40 | 12 |
| | | | Chronic Obstructive Pulmonary Disease (COPD) | J44.9 | 3 |
| | | | Acute Respiratory Distress Syndrome | J80 | 1 |
| K00-K93 | K | 7 | Peptic Ulcer | K27 | 2 |
| | | | Dyspepsia | K30 | 5 |
| | | | Hematemesis | K92.2 | 1 |
| L00-L99 | L | 1 | Drug Eruption | L27 | 1 |
| N00-N99 | N | 4 | Chronic Kidney | N18 | 1 |
| | | | CKD Stage 5 | N18.5 | 1 |
| | | | Kidney Insufficiency | N19 | 1 |
| | | | Urinary tract infection | N39 | 1 |
| R00-R99 | R | 36 | Haemoptysis | R04.2 | 1 |
| | | | Anosmia | R43.0 | 35 |
| | | | Ageusia | R43.2 | 32 |
| U00-U99 | U | 100 | Covid-19 | U00 | 100 |

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**Table 4.** Drug Use Profile

| No | Drug Type | ATC Code | Percentage of Patients (%) | Drugs Used | Percentage of Drug Use (%) |
|---|---|---|---|---|---|
| 1 | Antiviral | J05AB16 | 83 | Remdesivir | 47 |
| | | J05AX27 | | Favipiravir | 25 |
| | | J05A0H02 | | Oseltamivir | 15 |
| 2 | Antibacterial | J01FA10 | 91 | Azithromycin | 37 |
| | | J01MA14 | | Moxifloxacin | 49 |
| | | J01MA12 | | Levofloxacin | 40 |
| | | J01DC02 | | Cefuroxime | 2 |
| | | J01DD02 | | Ceftazidime | 11 |
| | | J01DD04 | | Ceftriaxone | 29 |
| | | J01DD12 | | Cefoperozone | 1 |
| | | J01DH02 | | Meropenem | 36 |
| 3 | Analgesic | N02BB02 | 91 | Metamizole | 18 |
| | | R05DA04 | | Codeine | 53 |
| | | N02BE01 | | Paracetamol | 32 |
| | | N05BA01 | | analysis | 8 |
| | | N02AA01 | | Morphine | 3 |
| | | N01AH02 | | Fentanyl | 2 |
| 4 | Antihistamine | R06AE07 | 37 | Setirizin | 37 |
| | | R06AA11 | | Dimenhydrinate | 2 |
| 5 | Corticosteroids | A01AC03 | 70 | Hydrocortisone | 40 |
| | | A01AC02 | | Dexamethasone | 32 |
| | | D07AA01 | | Methylprednisolone | 27 |
| 6 | Mucolytic | R05CB01 | 83 | Acetylcysteine | 83 |
| 7 | 5-HT3 Antagonist | A04AA01 | 20 | Ondansetron | 20 |
| 8 | Diuretic | C03CA01 | 36 | Furosemide | 36 |
| 9 | Antihypertensive | C09CA06 | 35 | Candesartan | 15 |
| | | C07AB07 | | Bisoprolol | 22 |
| | | C08CA05 | | Nifedipine | 1 |
| | | C08CA01 | | Amlodipine | 14 |
| | | | | nicardipine | 1 |
| | | C09CA03 | | Valsartan | 1 |
| 10 | Anticoagulants | B01AF01 | 45 | Rivaroxaban | 3 |
| | | B01AB01 | | Heparin | 35 |
| | | B01AB05 | | Enoxaparin | 14 |
| | | B01AX05 | | Fondaparinuks | 11 |
| 11 | Hypnosis and Anesthesia | N05BA12 | 47 | Alprazolam | 27 |
| | | N05BA01 | | Diazepam | 15 |
| | | N05CD08 | | Midazolam | 5 |
| 12 | Antiplatelet | B01AC04 | 14 | Clopidogrel | 13 |
| | | | | aspirin | 3 |
| 13 | Antianginal | C01DA08 | 11 | ISDN | 11 |
| | | C08CA04 | | nicardipine | 1 |
| | | C01EB15 | | myozidine | 1 |
| 14 | Hypolipidemic | C10AA05 | 33 | Atorvastatin | 9 |
| | | C01AA07 | | Rosuvastatin | 23 |
| | | C10AB04 | | Gemfibrozil | 3 |
| | | C10AB05 | | Fenofibrate | 2 |
| 15 | Antidiabetic | | 9 | Levemir | 9 |
| | | A10BF01 | | Acarbose | 2 |
| 16 | Anti-ulcer | A02BC03 | 76 | Lansoprazole | 8 |
| | | A02BC02 | | Pantoprazole | 62 |
| | | A02BC01 | | Omeprazole | 11 |
| | | A02BX02 | | Sucralfate | 9 |
| 17 | Supplement | | 85 | Gabaxa/Amino acids | 8 |

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| | | | | | |
|---|---|---|---|---|---|
| | | | | Vipalbumin | 10 |
| | | | | Sancoidan | 2 |
| | | | | Curcuma | 57 |
| | | | | Methylcobalamin | 5 |
| | | | | Hepamax | 11 |
| | | | | Synbiotic | 20 |
| | | | | Prorenal | 3 |
| 18 | Vitamin | A11GA | | Vitamin C | 78 |
| | | A11CC | | Vitamin D | 26 |
| | | | 89 | Furamin | 12 |
| | | | | Zegavit | 62 |
| | | | | Becom C/Z | 23 |
| 19 | Anti-Guat | M04AA01 | 4 | Allopurinol | 4 |
| 20 | Vertigo | | 2 | Betahistine Melisat | 2 |
| 21 | Psychosis and Similar Disorders | N05AD01 | 1 | Haloperidol | 1 |
| 22 | Hemostatic and Antifibrinolytic | B02AA02 | 6 | Tranexamic Acid | 6 |
| 23 | Anesthetic | L01XX11 | 6 | Tramus | 6 |
| 24 | Urinary and Genital System | V03AE07 | 2 | Aminoral | 2 |
| 25 | Shock and Hypotension | C01CA07 | 2 | Dobutamine | 2 |
| 26 | Tricyclic Antidepressants | N06AA09 | 2 | Amitriptyline | 2 |
| 27 | Nausea and vertigo | C10AB05 | | Fenofibrate | 2 |
| | | A03FA01 | 2 | Metoclopramide | 5 |
| | | A03FA03 | | Domperidone | 1 |
| 28 | Laxative | A06AB02 | 8 | Dulcolac | 8 |
| 29 | Cardiac Glycoside | | 2 | Fargoxin | 2 |
| 30 | Antiasthma and Bronchodilators | A07EA06 | 8 | Symbicort | 1 |
| | | | | Ventolin | 3 |
| | | R03DA05 | | Aminophylline | 5 |
| 31 | Immunoglobulins | | 3 | IVIG | 2 |
| | | J06BA | | Intract | 3 |

**Table 5.** Profile of DDD/100 Days of Hospitalization

| ATC Code | Antibiotics | DDD in WHO | Number of grams | DDD/100 days in patient | Length of Days of Antibiotic Use (Days); Mean |
|---|---|---|---|---|---|
| J01FA10 | Azithromycin | 0,3 | 99,5 | 26,26 | 185; 5 |
| J01MA14 | Moxifloxacin | 0,4 | 98,8 | 19,56 | 98; 2 |
| J01DD04 | Ceftriaxone | 2 | 248 | 9,82 | 73; 2,5 |
| J01DD12 | Cefoperazone | 4 | 8 | 0,16 | 8; 8 |
| J01MA12 | Levofloxacin | 0,5 | 159,75 | 25,30 | 213; 15,3 |
| J01DD02 | Ceftazidime | 4 | 138 | 2,73 | 69; 6,3 |
| J01DH02 | Meropenem | 3 | 510 | 13,46 | 170; 4,7 |
| J01DC02 | Cefuroxime | 3 | 6 | 0,16 | 6; 6 |
| | Total | | 1268,05 | 97,44 | 822 |

Antibiotics are a class of drugs used in the treatment of COVID-19 patients. The antibiotics use activity was assessed from the accuracy of the antibiotic's selection. According to the 3rd Edition of Covid-19 Management Manual, it is explained that the recommended antibiotic therapy is Azithromycin or Levofloxacin when there is an indication of bacterial infection (PDPI et al., 2020). The assessment of accuracy in the antibiotic's selection refers to the initial diagnosis of Covid-19 patients. Therefore, if there are no discrepancies such as the addition of other types of antibiotics, additional antibiotic therapy is maybe aimed at complications. According to the manual, the range of antibiotic use is 5-7 days (Gennaro et al., 2020).

The choice of antiviral therapy according to the Manual is Favipiravir and Remdesivir. Favipiravir is prescribed for the treatment of Covid-19. The use of this antiviral is limited to the treatment of cases that do not

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improve with other antivirals. This drug is currently used as a test drug for Covid-19 (Lapostolle et al., 2020). The use of antivirals as therapy is used to suppress the virus development, not to destroy the entire virus. The correct choice of antiviral depends on the patient's diagnosis and the choice of the antiviral prescribed. Antiviral selection profiles were compared with those listed under the Covid-19 Management Manual 3rd Edition. The appropriate antivirus, according to the Manual, was Favipiravir or Remdesivir (PDPI et al., 2020).

## CONCLUSION

Data shows that men are more exposed to Covid-19 than women. The condition of patients at the time of hospital admission was 44% moderate and 56% severe. Meanwhile, the condition of patients with moderate degree using antivirals and antibiotics at the time of hospital discharge was 84.1% recovered and 88.64% in improvement state. Meanwhile, the condition of patients with severe degrees using antivirals and antibiotics was 58.93% recovered and 58.92% in improvement state.

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## AUTHOR CONTRIBUTIONS

Conceptualization, R.Y.; Software, V.Y.T.; Methodology, F.H.; Validation, F.H., R.K.; Formal Analysis, V.Y.T.; Investigation, F.H., R.K.; Resources, V.Y.T., A.K.J.; Data Curation, V.Y.T.; Writing - Original Draft, V.Y.T.; Writing - Review & Editing, F.H., R.K., A.K.J.; Visualization, V.Y.T.; Supervision, F.H., R.K., A.K.J.; Project Administration, V.Y.T., A.K.J.; Funding Acquisition, V.Y.T.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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# Direct Medical Cost Analysis of Dengue Patients: A Retrospective Study

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***Abstract***

***Background****: Dengue infection is an arboviral disease that could lead to severe illness. The incidence of severe dengue will impact patients' financial aspects. As an important clinical feature, platelet level on admission day might contribute to the direct medical cost.* ***Objective****: This study aimed to examine the direct medical cost among patients with dengue fever (DF) or dengue hemorrhagic fever (DHF) in Yogyakarta, Indonesia.* ***Methods****: This study was a retrospective study conducted in two private hospitals in Yogyakarta, Indonesia. Participants included in this study were hospitalized patients diagnosed with DF or DHF. Data were extracted from medical records and finance departments in each hospital.* ***Results****: Among 174 dengue patients included in this study, the mean age of DHF patients (18.44 ± 14.87) was lower than in DF patients (23.47 ± 18.99). Patients with DHF (4.88 days) also showed prolonged hospitalization compared with DF (4.18 days) patients. In terms of medical cost, DHF patients need a higher dengue treatment cost, which is about 329.74 USD compared to 220.68 USD for DF patients in hospital 2. Mainly related to room charges, which increased by 9.48% in hospital 1 and 66.28% in hospital 2 compared with DF patients. Furthermore, DHF patients with thrombocytopenia on the first day of admission present higher medical costs in terms of laboratory fees (36.08%), medicine (18.17%), and total medical costs (9.84%).* ***Conclusion****: Severe form of dengue will contribute to economic burden. Therefore, prevention and adequate treatment are essential to reduce the prognosis of severe dengue and higher medical cost.*

***Keywords****: dengue, infection, medical cost*

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## INTRODUCTION

Dengue is a mosquito-borne viral infection caused by four dengue virus serotypes (DENV-1 to DENV-4). This infection, an arboviral disease, has different manifestations in each patient due to the host immunity or the virus serotype (Khan et al., 2020). Based on the manifestations, dengue infection could be divided into an undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), or other severe forms of dengue due to plasma leakage, which leads to dengue shock syndrome (DSS) and severe organ involvement (Pang et al., 2017; World Health Organization, 1997).

The incidence of infection and disability-adjusted life-years due to dengue increased in the past two decades. Furthermore, the high incidence of dengue in the world suggests that this infectious disease has become a significant public health challenge (Zeng et al., 2021). Data from Indonesia showed that the annual fatality rate declined by half in each decade. However, DHF incidence increases with incidence peaks every 6-8 years (Harapan et al., 2019). Moreover, coronavirus disease 2019 (Covid-19) became a new pandemic in 2019 that occurred globally and impacted health care systems worldwide. Dengue incidence in the Covid-19 pandemic seems to decrease during 2020 and 2021 (World Health Organization, 2022). Recently, the report of dengue incidence in Covid-19 in each country still varies. Some regions (such as Guangzhou, China and Sri Lanka) reported decreasing dengue cases (Jiang et al., 2021; Liyanage et al., 2021). However, other regions experienced an increased incidence of dengue during the Covid-19 pandemic (Lim et al., 2021; Plasencia-Dueñas et al., 2022).

The experience of dengue in patients could be related to reduced quality of life and financial problems (Elson et al., 2020; Panmei et al., 2019). In 2013, the global burden of disease study mentioned that there were 58.40 million symptomatic dengue virus infections, with 13.586 fatal cases, and the total annual international cost of dengue illness was 8.9 billion US$ (Shepard et al., 2016). The other previous study in India presented that the dengue treatment reached 2.16 million US$, which was lower than the cost of prevention (8.3 million US$). Furthermore, the total cost of dengue reached 38 million US$ (Nujum et al., 2020). A previous systematic review in 2019 stated that the dengue burden still significantly impacts the economic situation in particular countries with specific socioeconomic characteristics and similarities in health management systems (Oliveira et al., 2019).

According to a previous study, DHF patients have relatively high direct and indirect medical costs (due to productivity loss) due to the severity (Supadmi et al., 2019). Another study found that DHF patients require more extended hospitalization than DF patients (Faridah et al., 2022), implying that DHF medical costs will rise. Another factor contributing to increased direct medical costs is crucial clinical aspects of severe dengue, such as platelet count. As a result, this study aims to look at the direct medical costs of dengue patients of varying severity (DF or DHF) in Yogyakarta, Indonesia.

## MATERIALS AND METHODS

This study was a retrospective study conducted in two private hospitals in Yogyakarta, Indonesia. Participants included in this study were hospitalized patients diagnosed with dengue (dengue fever, dengue hemorrhagic fever, or dengue shock syndrome) using ICD code A90/A91. The data were randomly collected from 2018 to 2020. The sample size was calculated using the formula: Expected proportion of patients with dengue fever (p) = 0.33 based on the ratio of dengue patients aged 5-14 years old = 33%, expected precision = 0.1 and confidence interval at 95% (Pourhoseingholi et al., 2013; Rafikahmed et al., 2021). The minimum sample size was about 85.

$$n = \frac{Z^2_{1-\alpha/2}pq}{d^2}$$

Z1-α/2 = 1.96
p = 0.33
q = 0.67
d = 0.1

This study was approved by Health Research Ethics Committee of *Fakultas Kedokteran dan Ilmu Kesehatan Universitas Muhammadiyah Yogyakarta* (Ref : 063/EC-KEPK FKIK UMY/XII/2020) and Ethics Committee Approval from PKU Muhammadiyah Yogyakarta Hospital (Ref : 00101/KT.7.4/III/2021).

Classification of dengue manifestation was divided into undifferentiated fever, dengue fever, and dengue hemorrhagic fever. The definition of dengue fever is an acute febrile illness with two or more manifestations such as headache, myalgia, rash, hemorrhagic manifestations, or leukopenia. However, dengue haemorrhagic fever is fever with hemorrhagic manifestations (positive tourniquet test, petechiae, bleeding from the mucosa, gastrointestinal tract, or melena), thrombocytopenia (<100,000 cells per $mm^3$), and the sign of plasma leakage which manifested by

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rising hematocrit, pleural effusion, ascites, or hypoproteinemia (World Health Organization, 1997).

Data were extracted from medical records about sociodemographic and clinical features using a data extraction form. Sociodemographic data includes age, gender, education, mode of payment, room of stay and duration of hospitalization. Furthermore, each hospital's finance department obtained data on direct medical costs. The direct medical cost was defined as room stay charges, cost of laboratory tests, medicine, service fee, and a total of payment based on the hospital perspective.

Analysis of the data was performed using SAS 9.4. The characteristics and clinical features of the patients were described descriptively with percentages; however, age and length of hospitalization were summarized as mean and standard deviation (SD). A direct medical cost between DF and DHF was analyzed using the Mann-Whitney test or t-test, and the significance level was set at P-value < 0.05.

## RESULTS AND DISCUSSION

A total of 174 dengue patients were screened and included in this study, which are 85 patients diagnosed with DF and 89 patients with DHF. Of the total 174 dengue patients, the mean age was 20.9 years (± SD 17.14) which the mean age of DHF patients was lower than in DF patients. This data also represents the group of age, in which 37.65% of DF patients were adults (21 to 60 years old); however, 40.45% of DHF patients were children below 12 years old. Other characteristics of our respondents are the majority having lower than senior high school for education (95.97%), male gender (57.47%), and using government insurance (72.41%). The average stay in the hospital for all patients was 4.53 days (+ SD 1.5), and the mean stay in the hospital was longer in DHF patients than in DF patients (Table 1).

Regarding the age with the incidence of dengue, the results of this study were similar to previous studies. Paediatric patients significantly have more risk of developing a severe form of dengue than adult patients (Hegazi et al., 2020). A previous study in Brazil summarized that complicated dengue, DHF, or DSS were higher in younger age classes than in adults (Burattini et al., 2016). However, a severe form of dengue could occur at an older or younger age. Older age patients who suffered severe dengue and were admitted to the Intensive Care Unit (ICU) were correlated with multiple comorbidities (including hypertension, diabetes mellitus, and chronic kidney disease) and high case-fatality rate (Hsieh et al., 2017). Meanwhile, pediatric patients tend to present hypovolemic shock or DSS, and daily monitoring of platelet count can help identify patients that are at high risk of DSS (Lam et al., 2017).

**Table 1.** Characteristics of dengue patients

| | Dengue Fever (n = 85) | Dengue Hemorrhagic Fever (n = 89) | Total |
|---|---|---|---|
| Age (years), mean±SD | 23.47 + 18.99 | 18.44 + 14.87 | 20.90 + 17.14 |
| < 12 years old | 23 (27.06) | 36 (40.45) | 59 (33.91) |
| 12 - 21 years old | 23 (27.06) | 24 (26.97) | 47 (27.01) |
| 21 - 60 years old | 32 (37.65) | 27 (30.34) | 59 (33.91) |
| > 60 years old | 7 (8.24) | 2 (2.25) | 9 (5.17) |
| Male Gender | 50 (58.82) | 50 (56.18) | 100 (57.47) |
| Education | | | |
| < Senior High School | 82 (96.47) | 85 (95.51) | 167 (95.97) |
| > Senior High School | 3 (3.53) | 4 (4.49) | 7 (4.02) |
| Payment | | | |
| Indonesia's National Health Insurance | 59 (69.41) | 67 (75.28) | 126 (72.41) |
| Self-paid | 21 (24.71) | 16 (17.98) | 37 (21.26) |
| Others | 5 (5.88) | 6 (6.74) | 11 (6.32) |
| Length of Hospitalization (days), mean+SD | 4.18 + 1.28 | 4.88 + 1.62 | 4.53 + 1.50 |
| Room | | | |
| 3 | 29 (34.12) | 20 (22.47) | 49 (28.16) |
| 3A | 9 (10.59) | 7 (7.87) | 16 (9.20) |
| 2 | 19 (22.35) | 33 (37.08) | 52 (29.89) |
| 1 | 17 (20) | 12 (13.48) | 29 (16.67) |
| VIP | 8 (9.41) | 13 (14.61) | 21 (12.07) |
| VVIP | 3 (3.53) | 4 (4.49) | 7 (4.02) |

SD : Standard Deviation

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**Table 2.** Clinical features of dengue patients

| Symptom | Dengue Fever (n = 85) | Dengue Hemorrhagic Fever (n = 89) | Total |
|---|---|---|---|
| Headache | 26 (30.59) | 30 (33.71) | 56 (32.18) |
| Rash | 2 (2.35) | 1 (1.12) | 3 (1.72) |
| Fatigue | 0 | 1 (1.12) | 1 (0.57) |
| Diarrhea | 7 (8.24) | 3 (3.37) | 10 (5.75) |
| Gum Bleeding | 0 | 1 (1.12) | 1 (0.57) |
| Nausea/Vomiting | 48 (56.47) | 46 (51.69) | 94 (54.02) |
| Abdominal Pain | 16 (18.82) | 15 (16.85) | 31 (17.82) |
| Bone Pain | 6 (7.06) | 2 (2.25) | 8 (4.60) |
| Gastric Bleeding | 0 | 1 (1.12) | 1 (0.57) |
| Hemoptysis | 1 (1.18) | 6 (6.74) | 7 (4.02) |
| Thrombocytopenia | 32 (37.65) | 48 (53.93) | 80 (45.98) |
| High Hematocrit | 41 (48.24) | 37 (41.57) | 78 (44.83) |

Prolonged hospital stays are also represented in DHF patients in this study. This result was in line with previous research that fatal cases of DHF patients had prolonged hospital stays than controls (Thein et al., 2013). Other predictors than DHF, such as an elevated liver enzyme, prolonged prothrombin time (PT) or activated partial thromboplastin time (aPTT) and the presence of multiple organ disorders, are also known to contribute to prolonged hospitalization (Mallhi et al., 2017).

Dengue hemorrhagic fever is a severe form of dengue. Data from Table 2 represent the clinical features of dengue patients, and the top three features are nausea/vomiting, headache, and abdominal pain. This result was similar to the previous result that dengue patients included nausea/vomiting, headache, and abdominal pain in the top five clinical characteristics. However, in contrast to the previous study, nausea/vomiting and abdominal pain showed a higher percentage in DF patients than in DHF patients due to a small number of patients (Jayarajah et al., 2020).

Other presentations, gum and gastric bleeding, showed only in DHF patients. Gum/gingival bleeding includes minor bleeding that usually happens in a critical phase of dengue. Bleeding in dengue patients could be associated with coagulation abnormalities and vascular leak syndrome. Moreover, severe haemorrhages are shown by gastrointestinal bleeding or severe vaginal bleeding (Azeredo et al., 2015). However, due to the small sample size in this study, the number of patients suffering from bleeding was very small (Table 2).

Although the mechanism of thrombocytopenia and bleeding in dengue patients is not fully understood, it is known that platelets play a role in thrombus formation. The decreasing number of platelets in dengue could be associated with dengue severity and bleeding in dengue patients (Wilder-smith et al., 2019). Furthermore, anti-platelet antibodies in patients with dengue virus (DENV) cause platelet lysis or dysfunction and contribute to some clinical features of DHF (Azeredo et al., 2015). Our study also revealed that patients with low platelet count on on-admission day were significantly higher in DHF patients (53.93%) rather than in DF patients (37.65%) (p-value=0.0312) (Table 2).

Figure 1 shows the direct medical cost percentage compared with the total cost for DF and DHF patients, while Table 3 summarizes the direct medical cost data comparison between DF and DHF at two private hospitals in Yogyakarta. The percentage of the cost was higher in room charge (26%) and medicine cost (26%) for DF patients, while in DHF patients, the higher percentage of the price was in room charge (31%) and laboratory cost (25%). In sub-analysis data between two different hospitals, our study result showed that DHF patients need a higher cost of dengue treatment, which is about 329.74 USD, compared to 220.68 USD for DF patients in hospital 2. This is especially related to room charges, which are higher at 9.48% in private hospital 1 and 66.28% in private hospital 2. Higher costs also showed in the service fee of DHF patients, which were higher at 8.65% in private hospital 1 and higher at 16.87% in private hospital 2. This result was statistically significant in private hospital 2 (p-value=0.0028 for room charge and p-value=0.0498 for service fee), and although it present insignificant in private hospital 1, the trend was similar in that DHF patients need extra payment for room charges and service fees. A previous study in India also revealed that adults or paediatrics

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with severe dengue need higher costs than dengue patients with or without warning signs (Panmei et al., 2019). As an expectation, a severe form of the disease will increase the medical cost, such as hospitalization, laboratory fee, and fee for professional services. Based on the data from private hospital 2, the cost for laboratory testing and comprehensive treatment also showed significant differences between DF and DHF patients, but the result showed differences in private hospital 1. This study showed that in private hospital 1, laboratory costs, medicine, and treatment total were higher in DF patients than in DHF patients. This happened due to the distribution of room class 28% of DF patients stayed in class 1 and VIP, while only 22% of DHF patients stayed in the same class. The difference in classroom distribution impacts some costs that are higher in DF than in DHF patients.

Platelet is one of the factors that contribute to the severity of dengue. Bone marrow depression induced by DENV causes thrombocytopenia. Thrombocytopenia in dengue patients increases the risk of bleeding manifestation and other severe complications. However, not all patients present thrombocytopenia, a previous study in Brazil showed that thrombocytopenia happened in 40.3% of respondents (Castilho et al., 2020). Platelet count during the febrile phase can be used as a risk factor for developing shock syndrome, and monitoring of the changes in platelet count over time is also related to the development of shock syndrome (Lam et al., 2017). Hence, platelet counts on admission day could be related to dengue prognosis and higher medical costs. Considering the previous results that DHF needs extra payment for medical costs, data in Table 4 emphasized that DHF patients with low platelet levels present higher medical costs (236.11 USD) than DHF with normal platelet patients (214.95 USD). The significant result showed higher laboratory cost, medicine, and total medical cost (p-value=0.0003, 0.0033, and 0.0106, respectively). Although the cost for room charges in DHF presents insignificant results, the average of room charges was higher in DHF with low platelet than normal platelet count.

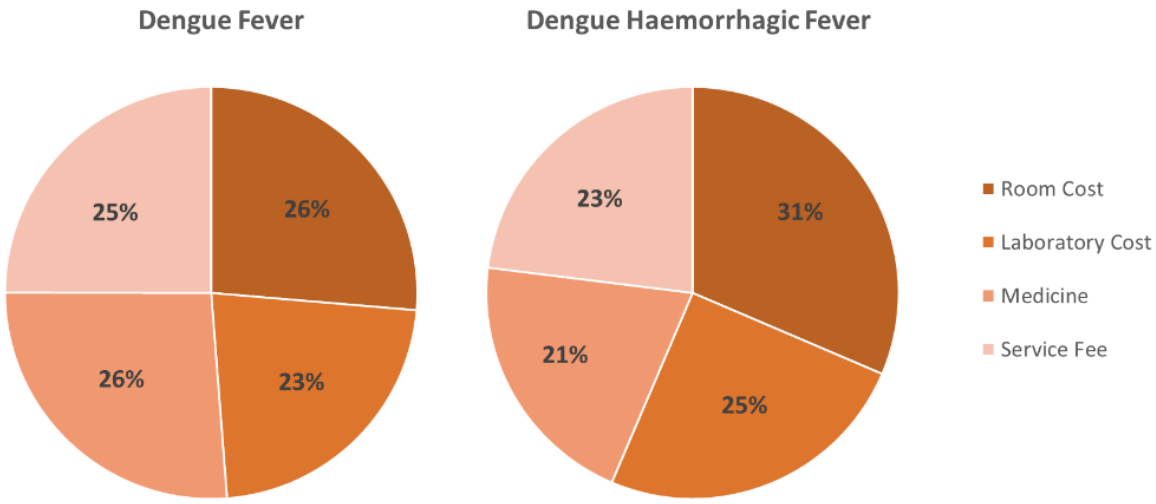


**Figure 1**. The percentage of direct medical cost for dengue fever and dengue hemorrhagic fever patients

**Table 3.** Direct medical costs in US Dollars (USD) for dengue fever and dengue hemorrhagic fever in different private hospitals in Yogyakarta

| | Private Hospital 1 (n = 100) | | | Private Hospital 2 (n = 74) | | |
|---|---|---|---|---|---|---|
| | DF (n = 50) Mean ± SD | DHF (n = 50) Mean ± SD | P-value* | DF (n = 35) Mean ± SD | DHF (n = 39) Mean ± SD | P-value* |
| Room Cost | 36.80 ± 22.21 | 40.29 ± 19.28 | 0.0992 | 66.58 ± 61.15 | 110.71 ± 94.75 | **0.0028** |
| Laboratory Cost | 33.98 ± 20.76 | 29.07 ± 13.16 | 0.7987 | 52.91 ± 41.90 | 91.79 ± 105.89 | **0.0064** |
| Medicine | 43.64 ± 41.81 | 25.29 ± 26.53 | **0.0032** | 56.41 ± 38.23 | 73.71 ± 72.94 | 0.6729 |
| Service Fee | 47.00 ± 14.76 | 51.07 ± 13.12 | 0.1392 | 45.80 ± 15.53 | 53.53 ± 17.58 | **0.0498** |
| TOTAL | 161.42 ± 68.93 | 145.73 ± 48.28 | 0.3683 | 220.68 ± 107.92 | 329.74 ± 191.44 | **0.0087** |

Exchange rate during the study period: 14499 IDR to 1 USD
*Calculated using the Mann Whitney Test

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**Table 4.** Subgroup analysis of direct medical costs (in US Dollars (USD)) for dengue fever and dengue hemorrhagic fever based on on-admission day platelet

| | DF (n=85) | | | DHF (n=89) | | |
|---|---|---|---|---|---|---|
| | Thrombocytopenia (n=32) Mean ± SD | Normal Platelet (n=53) Mean ± SD | P-value* | Thrombocytopenia (n=48) Mean ± SD | Normal Platelet (n=41) Mean ± SD | P-value* |
| Room Cost | 40.22 ± 37.74 | 54.40 ± 48.31 | 0.1380 | 71.38 ± 65.31 | 70.88 ± 81.78 | 0.1857 |
| Laboratory Cost | 54.35 ± 43.39 | 34.19 ± 20.43 | **0.0105** | 64.42 ± 74.22 | 47.34 ± 79.91 | **0.0003** |
| Medicine | 47.19 ± 32.05 | 49.93 ± 45.30 | 0.6275 | 50.06 ± 42.09 | 42.36 ± 71.32 | **0.0033** |
| Service Fee | 47.98 ± 12.47 | 45.61 ± 16.39 | 0.3295 | 50.25 ± 14.36 | 54.37 ± 15.99 | 0.2033 |
| TOTAL | 189.75 ± 88.09 | 183.45 ± 94.03 | 0.5770 | 236.11 ± 142.91 | 214.95 ± 178.81 | **0.0106** |

Exchange rate during the study period: 14499 IDR to 1 USD
*Calculated using the Mann-Whitney Test

Considering the different health management settings and geographical situations in other countries, ten years ago, the costs for dengue outbreaks in Indonesia, Peru, and Vietnam were 6.75 million US $, 4.5 million US$ and 12 million US$, respectively. The cost components were vector control, surveillance, information, education and communication, and direct and indirect medical costs (Stahl et al., 2013). Previously study in Indonesia mentioned that the aggregate cost of dengue was 73% higher than the estimation. The study also presented that dengue costs in hospital settings and non-fatal cases were the highest compared to ambulatory and not medically attended settings (Wilastonegoro et al., 2020). In addition, a previous study in Taiwan compared dengue's economical cost and disease burden during epidemic and non-epidemic. The cost in the epidemic year was 12.3 times higher than in the non-epidemic year, and the highest was the cost of hospitalization (86.09%). However, the drug cost was the lowest (0.03%) (Luh et al., 2018).

Based on previous information, analysis of direct medical costs for hospitalized dengue patients is essential. This study highlights that severity of dengue will impact the economic aspect. Thus, identifying the high risk of severe dengue patients is potentially valuable for preventing mortality and reducing direct medical cost. Results of this study showed similar to other studies, in which severe dengue patients had higher costs for treatment than different grades of dengue (Panmei et al., 2019; Vieira Machado et al., 2014). The key to successful treatment and lowering the treatment cost correlates with a better triage system for hospitalization and the availability of technical or laboratory support that is useful for predicting high-risk dengue patients (Bajwala et al., 2019; Ministry of Health Republic of Indonesia, 2021).

The design of the present study was limited. This was a retrospective study with a small number of samples in each hospital or group. Therefore, we could not find consistent results between the two hospitals. Additionally, the limitation of the retrospective study was incomplete data, so some variables related to medical cost cannot be evaluated in this study. Thus, the indirect costs associated with the loss of productivity, such as travel, food, and the absence of families or caregivers from school or activities, cannot be analyzed. This study only estimated direct medical costs of hospitalization.

## CONCLUSION

In conclusion, a severe form of dengue will contribute to the economic burden. DHF patients with a low platelet level on the first day of admission showed need higher cost on specific variables than DHF patients with normal platelets. Hence, prevention and adequate treatment are essential to reducing severe dengue prognosis and higher medical costs.

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## AUTHOR CONTRIBUTIONS

Conceptualization, W.S., D.A.P.; Methodology, H.D., I.N.F.; Validation, W.S., D.A.P.; Formal Analysis, I.N.F., H.D.; Investigation, H.D., D.A.P., W.S., I.N.F.; Resources, I.N.F., T.L.S., R.A.N.; Data Curation, T.L.S., R.A.N.; Writing - Original Draft, I.N.F., W.S., D.A.P. ; Writing - Review & Editing, I.N.F., W.S., D.A.P.; Supervision, D.A.P., W.S.; Project Administration, H.D., I.N.F., W.S., D.A.P.; Funding Acquisition, W.S., D.A.P.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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# Acute and Subchronic Toxicity of Indonesian House Dust Mites (IHDM) Allergenic Extract for Asthma Allergy Immunotherapy

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***Abstract***

***Background****: In developing a pharmaceutical product, it is necessary to conduct pre-clinical and clinical trials to ensure its safety and effectiveness. The toxicity test is conducted to assess the safety of a substance to determine its toxic effect of the substance.* ***Objective****: This study aims to determine the acute and subchronic toxicity of administering IHDM allergenic extract using experimental animal models.* ***Methods****: Female BALB/c mice and female and male Wistar rats were used as experimental animal models. While the IHDM allergenic extract was used with the level of Der p1 is 11.3-26.6 ng/mL and was administered by intravenous route. The acute toxicity test was carried out for 14 days on four different dose groups of experimental animals. The subchronic toxicity test was carried out for 28 days using three other dose groups of experimental animals.* ***Results****: The administration of a single dose of IHDM allergenic extract at various doses did not cause mice behaviour changes, and no death was shown in each group. Likewise, there was no change in the principal organs by macroscopic observations. Meanwhile, administering IHDM allergenic extract at repeated doses for 28 days could show signs of toxicity. The symptoms were shown in the histopathological structure of the liver, kidney, and heart organs.* ***Conclusion****: It can be concluded that the IHDM allergenic extract is safe for single-dose administration but shows toxic signs when given in repeated doses. Further tests are needed for 90 days of subchronic toxicity and satellite testing.*

***Keywords****: acute toxicity, subchronic toxicity, IHDM safety, asthma allergy, neglected disease*

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## INTRODUCTION

Asthma is a chronic lung disease that has affected approximately 300 million people worldwide, and this disease causes about 346,000 deaths annually (Sisson et al., 2018). According to the Global Burden of Disease, it was estimated that 420,000 people died from asthma, and there were more than 1,000 deaths per day in 2016 (GAN, 2018). This disease can attack everyone and is one of the leading causes of early dying and can also cause a decrease in the quality of life in all populations (GAN, 2018). Asthma is estimated to affect about 339 million people worldwide. Asthma is one of the leading causes of disability and mortality for sufferers (GAN, 2018).

Asthma is generally characterized by chronic inflammation of the airways. The respiratory symptoms are wheezing, shortness of breath, chest tightness, shortness of breath, and cough that varies periodically with persistently restricted expiratory airflow. The symptoms can arise due to several causes, such as sporting activities, exposure to allergens or irritants, changes in weather, or viral infection of the respiratory tract. These symptoms may disappear spontaneously during the time, but symptoms of asthma exacerbation may also threaten the sufferer's life (GINA, 2020).

Allergic asthma involves cell inflammation, including eosinophils, mast cells, T lymphocytes, neutrophils, and macrophages. There is a bias in the immune response in asthma, which includes the infiltration of Helper 2 T cells into the lungs and the presence of secretions cytokines (IL-4, IL-5, IL-9, IL-13, and IL-33). This cytokine is a sign of eosinophil inflammation, leading to the production of specific IgE allergens and the presence of airway hyperresponsiveness (AHR), and the release of inflammatory mediators such as eosinophils, mast cells, T and lymphocytes, neutrophils (Balkrishna *et al.*, 2020).

House Dust Mites (HDM) is one of the most common causes of respiratory allergies globally. It can be found in children, adolescents, and adults—high exposure to mite allergens. Asthma patients sensitive to HDM can trigger bronchospasm and increased bronchial hyperreactivity. At the same time, cessation of exposure to allergens can relieve these symptoms (Zuiani & Custovic, 2020). *Dermatophagoides pteronyssinus* (Der p) has at least 23 allergens predicted to contribute to the sensitization process through proteolytic activity, activating the body's innate immune cells underlies the type 2 adaptive immune response (Hesse *et al.*, 2020). Currently, allergen immunotherapy is one of the treatments for allergic asthma. Allergen immunotherapy is a treatment strategy for IgE-mediated allergic disease. According to Drazdauskaitė *et al.* (2021), allergen immunotherapy is the only therapy that can modify the immunological processes underlying specific allergic asthma to immunotherapy. In recent years, it has been recognized that immunotherapy of HDM allergenic extract has been widely registered as a pharmaceutical product in allergic asthma (Eguiluz-gracia *et al.*, 2020).

In Indonesia, an HDM allergenic extract product was developed and is used as immunotherapy. In creating a pharmaceutical product, it is necessary to conduct pre-clinical and clinical trials to ensure its safety and effectiveness of a pharmaceutical product. Pre-clinical testing is carried out through two test stages, namely: effectiveness test and toxicity test. The effectiveness test is carried out to determine the effectiveness of a compound or substance. In contrast, the toxicity test is carried out to assess the safety of a compound or substance to determine the toxic effect of a compound or substance. Several studies found that allergen extracts had different allergenicity when they came from various regions and/or were developed with other manufacturers (Cheong *et al.*, 2009; Zimmer, Vieths & Kaul, 2016).

This is evidenced by several studies showing that the administration of HDM allergens with various variations causes differences in T cell responses, binding to IgE, and even the toxicity effect which may occur due to: differences in amino acid sequences in allergenic proteins, differences in raw materials, and differences in composition (Hales *et al.*, 2002; Weghofer *et al.*, 2008; Casset *et al.*, 2012). Therefore, this study will examine acute and subchronic toxicity in the desensitization of IHDM allergenic extract.

## MATERIALS AND METHODS

### Materials

#### *Allergenic extract*

The allergenic extract used is Indonesian house dust mites allergenic extract (Der p1 = 11.3-26.6 ng/mL). The allergenic extract was purchased from Dr Soetomo Regional Hospital (Surabaya, Indonesia). The allergenic extract was administrated by intravenous injection.

#### *Experimental animals*

Mice and rats were purchased from Pusat Veterener Farma, Ministry of Agriculture of The Republic of Indonesia (Surabaya, Indonesia). Healthy female

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BALB/c strain mice aged 6-8 with 20-25 g in weight, which required nulliparous non-pregnant, were used for the acute toxicity test. Healthy male and female Wistar strain rats aged 6-8 weeks with 250-300 grams. All experimental animals were adapted for at least a week. All animals were housed in ventilated cages with ad libitum access to water, a standard pelleted diet under controlled temperature conditions (23±2 oC), and a light cycle (12 hours light/dark). All animal procedures were carried out in compliance with the ethical committee of the Faculty of Veterinary Medicine, Universitas Airlangga, for the use of laboratory animals (Number: 2.KE.058.05.2021).

**Method**

***Acute toxicity test***

The acute toxicity test was done as the Guideline of Preclinical Toxicity Test from the National Agency for Food and Drug Control of The Republic of Indonesia and the Organisation for Economic Co-Operation and Development (OECD) Guideline. In the acute toxicity test, mice were divided into four groups, namely: the control group were given saline. Group 1 was given the Indonesian HDM with a dose of 0.13 mg. Group II was given the Indonesian HDM with a dose of 1.3 mg. Group III was given the Indonesian HDM with a dose of 2.6 mg. Each group consisted of 10 mice. The observation was done for the first 30 minutes, 4 hours, and 24 hours for 14 days. The mice were sacrificed on the 15th day.

***Subchronic toxicity test***

The subchronic toxicity test was done as the Guideline of Preclinical Toxicity Test from the National Agency for Food and Drug Control of The Republic of Indonesia and the OECD Guideline. In the subchronic toxicity test, rats were divided into three groups: the Control group were given saline. The Indonesian HDM was given to Group 1 at a dose of 0.09 mg. The Indonesian HDM was given to Group II at a dose of 0.9 mg. The observation lasted 28 days. On the 29th day, the mice were sacrificed.

***Parameter***

***Bodyweight observation***

In the acute toxicity test, the body weight of the mice was observed for 14 days. In the subchronic toxicity test, the body weight of the rats was observed for 28 days.

***Behavioural observation***

Changes in behaviour that can be visually observed are walking backwards or walking in circles and changes in the eyes, skin, and feet. In addition, it is also observed the presence of symptoms of toxicity that arise in the body of rats, such as the emergence of spots, sores, nasal discharge, and even death (Ningsih *et al.*, 2017).

***Organs' weight and gross pathology observation***

Animal organs (heart, liver, and kidney) were weighed and measured the size using millimetre block for the toxicity tests after the animals were sacrificed. The presence of changes in shape, colour, or size and visible lesions were also observed.

***Histopathological observation***

In the subchronic toxicity test, organs taken from animals are then fixed in a 10% formalin solution for one week. Then paraffin blocks are made and cut into 4 mm. After that, Haematoxylin & Eosin staining was performed on the specimens and observations were made using a microscope at 400 times magnification. Each organ was scored for lesions and then compared between the group.

***LD50 Calculation***

LD50 calculation is done using linear regression probit analysis with the help of the IBM SPSS Vers. 26 Software (New York, USA).

**Table 1.** The organs' histopathological toxicity scoring

| Organs | Description | Toxicity Score |
|---|---|---|
| **Heart** | Normal cell condition | 1 |
| | Mild lesion | 2 |
| | Moderate lesion | 3 |
| | Severe lesion | 4 |
| **Liver** | Normal cell condition | 1 |
| | Portal inflammation | 2 |
| | Degeneration parenchymatous or fibrotic | 3 |
| | Presence of necrosis | 4 |
| **Kidney (presence of lesions)** | < 25% | 1 |
| | 26 – 50 % | 2 |
| | 51 – 75 % | 3 |
| | 76 – 100 % | 4 |

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***Statistical data analysis***

Data of bodyweight was statistically analyzed using Two-Way ANOVA. Data of the organs' weight and histopathological toxicity score were statistically analyzed using One-Way ANOVA. All statistical data processing was processed using the Graph Pad Prism 9.0 program (California, USA).

## RESULTS AND DISCUSSION

***Acute toxicity test***

***Bodyweight observation***

The weighing of the mice was carried out every day. In the acute toxicity test, the body weight of mice was weighed every day for 14 days and then recorded for later analysis of changes in daily body weight in each group of experimental animals. Figure 1 shows the weight profile of mice in the acute test toxicity were weighed daily for 14 days. This picture shows no change or significant daily weight loss (Two-Way ANOVA, F(13.504)=1.191, P=0.2820). As for the dose group 3 at days 1-3 seen weight loss, but on the 4th day and subsequent weight gain. In the control group, doses 1 and 2 showed no change in weight that stands out daily.

***Behavioral observation***

No toxicity symptoms and death occurred during the test, so the probit analysis was not done.

***Organs' weight and gross pathology observation***

The heart, liver, and kidney were weighed and observed for the presence of changes in shape, color, or size and visible lesions of each group's organ.

In Figure 2 above, it can be seen that the liver size between the Control group, Group 1, Group 2, and Group 3, have been the same between groups, and does not appear differences in the colour of the liver organs between each group. Each animal's heart size group in the heart organ is known to have almost the same size between groups. In the kidney organs, it is known that there were no visible changes in colour between organs of each group, and visual size visually looks no different in each group.

It is known in Figure 3. It indicates no significant difference in organs' weight between each group (One-Way ANOVA, F(3,24)=2.236, P=0.1099 for the liver; F(3,24)=3.386, P=0.0214 for the heart; F(3,24)=1.750, P = 0.1836 for the kidney).

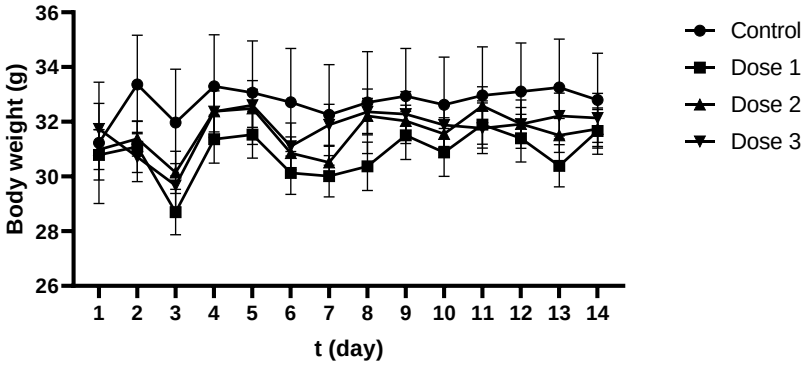


**Figure 1**. Bodyweight changes of mice used for acute toxicity test

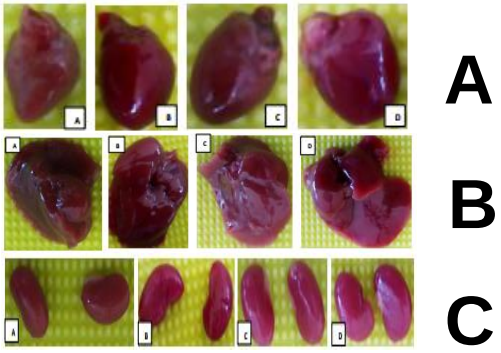


**Figure 2**. The visual appearance of the heart (A), liver (B), and kidney (C) organs for each group. (a) Control group; (b) Group I; (c) Group II; and (d) Group III

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***Subchronic toxicity test***

***Bodyweight observation***

The weighing of the rats was carried out every day for 28 days. Figure 4 shows a graphic profile of rats' body weight in each group in the subchronic toxicity test weighed daily for 28 days. The data obtained showed no significant change in body weight from each group each day (Two-Way Anova F(27,336)=0.5829 P=0.9540 for males; F(27,336)=0,2229 P>0.9999 for females).

***Behavioural observation***

In the subchronic toxicity test, two rats died during the 28-day observation period, a rat in Group I and a rat in Group II. These data indicate that IHDM allergenic extract administration repeatedly for 28 days can cause toxicity in experimental animals starting at a dose of 0.09 mg.

***Organs' weight and gross pathology observation***

The heart, liver, and kidney were weighed and observed for changes in shape, color, or size and visible lesions of each group's organ. In Figure 5, there appears to be no size difference between the Control group, Group I, and Group II in the rat liver. Then, there are no differences in colour, size, and shape for the heart organ when compared. In the kidney, the differences can be observed in each group. In the Control group, the colour is reddish-brown, Group 1 is reddish-brown but more faded, and Group 2 looks dark brown. These observations show that giving repeated-dose IHDM allergenic extracts can cause toxic effects on kidneys were observed changes visually in kidney color.

Based on the graph above, it was seen that there was no significant change in organs' weight between each group (One-Way Anova; F(2.6)=3.429, P=0.1016 for the liver; F(2.6)=0.3750, P=0.7023 for the heart; F(2,6)=0,2523, P=0,7849 for the kidney).

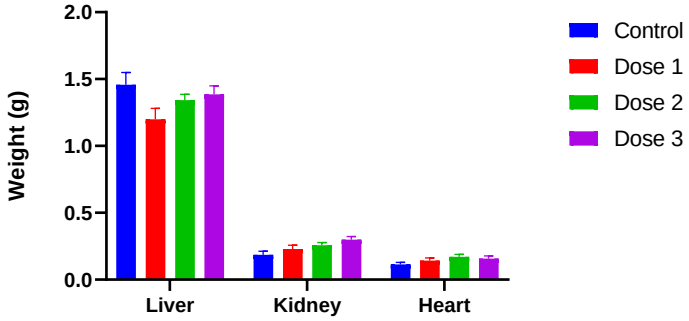


**Figure 3**. Each group's organs' weight profile of heart, liver, and kidney. Data are means ± SEM (n=10) *P<0.05 compared to control

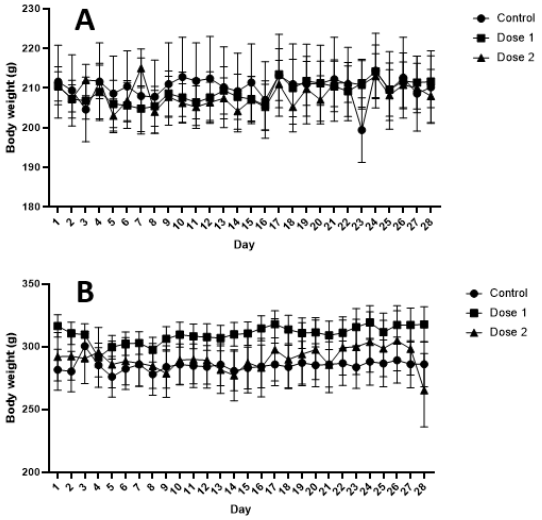


**Figure 4**. The bodyweight profile for each group of acute toxicity test. Male rats (A) and female rats (B). Data are means ± SEM (n=10) *P<0.05 compared to control

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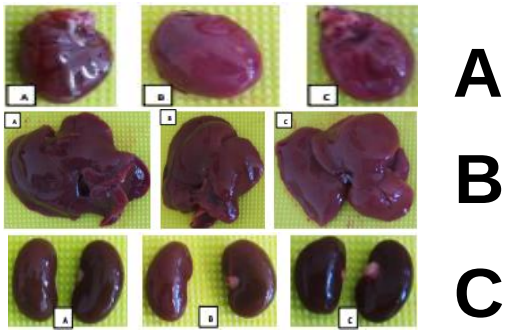


**Figure 5**. The visual appearance of the heart (A), liver (B), and kidney (C) organs for each group. (a) control group; (b) group I; and (c) group II

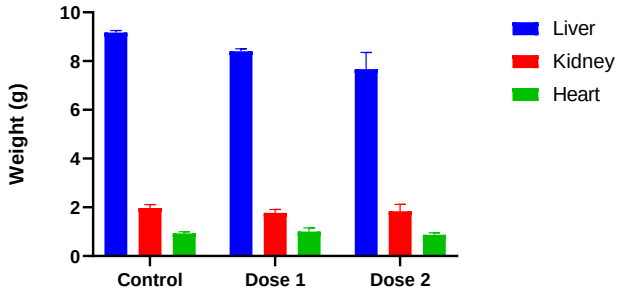


**Figure 6**. Each group's organs' weight profile of liver, heart, and kidney. Data are means ± SEM (n=10) *P<0.05 compared to control

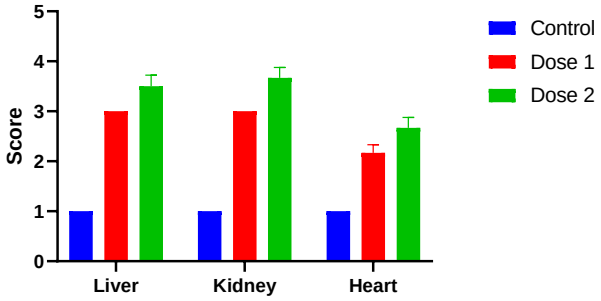


**Figure 7**. The organs' histopathological toxicity score profile of the rats. Data are means ± SEM (n=10) *P<0.05 compared to control

***Organs histopathological toxicity score***

It is known that administration of IHDM allergenic extract causes Histological changes in the liver, kidneys, and heart in the form of lesions on organs. Based on the graphic below, it is known that there was the difference in scoring scores in the liver, kidneys, and heart of experimental animals between groups. There is an increase in the score in test groups 1 and 2 compared to the control group. The greater the score obtained, the higher the score lesion or damage that occurs in the organ.

According to Ningsih et al. (2017), a change in body weight is one of the most accessible indicators visible. It is also an early indicator of the toxic effects of giving a sample of given test preparation. On the results of observations weighing in acute toxicity tests and subchronic toxicity during the trial period, not known to occur significant weight change between groups. The result found that animals' average body weight every day fluctuated, and generally no weight loss. It can be caused by feeding, where feeding will directly affect the weight of experimental animal bodies (Ningsih *et al.*, 2017).

Observation of liver, heart, and kidney organs was carried out in this study because the liver is an organ that acts as a site of metabolism drugs and toxic materials that enter the body and play a role in the system of blood flow to and from throughout the body through the

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system hepatic portal (Insani *et al.*, 2015). While the kidney has a volume high blood flow, carrying toxic cells through the tubules, and is an organ that produces urine, where urine is the major route of excretion of most toxicants (Makiyah & Tresnayanti, 2017). The heart is prone to abnormalities due to chemical compounds due to the number of mitochondria in the heart muscle relatively large numbers so that they are susceptible to cardiotoxicity. Another reason for choosing the three organs is because these three organs are involved in the metabolism of nutrients, drugs, and toxicants (Makiyah & Tresnayanti, 2017).

The liver is a portal circulation organ that transports compounds to be absorbed in the GIT. Metabolism toxic to the liver continuously results in toxicity liver, producing one or more reactive metabolites. Metabolites These reactive molecules bind macromolecular cells (such as proteins and lipids) irreversible so that it can cause loss of function of the macromolecule. One of the toxicity targets in the liver is the endoplasmic reticulum, which plays a role in protein synthesis in hepatocytes and is also the site of reactive metabolites of xenobiotics formed. It causes the endoplasmic reticulum to become susceptible to toxicant targets and produces injury by dilatation (Roberts *et al.*, 2000).

In the heart, mitochondria play an important role in susceptibility to cardiotoxicity. Cardiotoxicity is caused by oxidative stress, which causes the release of reactive oxygen species (ROS). This ROS release can stimulate lipid peroxidase and oxidative damage to mitochondria and myocyte cell membranes. Increased oxidative stress may also lead to the expression of transcription factors such as nuclear factor kappa B cell (NF-kB) and activation of the NLRP3 inflammasome, which results in increased release of pro-inflammatory cytokines myocardium as TNF- and IL-1β (Shaker *et al.*, 2018).

Several factors cause the kidneys to be susceptible to toxicity (Zhao et al., 2012). Kidneys are organs that play an essential role in maintaining the balance of body fluids and maintaining plasma volume and in, plasma volume and acid-base balance. Kidneys also detoxify organs and excrete toxic waste products of body metabolism (Ernawati, Witjahyo & Ismail, 2018). The toxic metabolism process in the kidney has the same way as solution metabolism serum, namely by passive glomerular filtration, passive tubular diffusion, and active tubular secretion. The kidneys require a large amount of ATP to maintain their transport function. ATP requirements high levels of this can cause the kidneys to be more susceptible to exposure to various toxins and hypoxic conditions (Zhao *et al.*, 2012; Ernawati *et al.*, 2018).

Further toxicity test studies, such as chronic toxicity tests, are conducted to determine the presence of toxic effects arising from the administration of long-term IHDM allergenic extract that has not been seen in this research. Then, the satellite group's evaluation shows the continuous subchronic effect of administering IHDM allergenic extract.

## CONCLUSION

Administration of a single dose of IHDM allergenic extract does not cause toxic effects characterized by the absence of mortality in experimental animals during the observation period and not found signs of toxicity in experimental animals. Repeated doses of IHDM allergenic extract can result in toxic effects starting at a dose of 0.09 mg, characterized by death in experimental animals and the difference between macroscopic and microscopic conditions of organs in the test group with the control group. Administration of IHDM allergenic extract dose of 0.09 mg given for 28 days can cause histopathological changes in the liver, kidney, and heart of experimental animals marked with the difference in scoring scores in the higher test group than the control group.

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## AUTHOR CONTRIBUTIONS

Conceptualization, J.K., M.T.; Methodology, J.K., M.T., A.S.B.; Validation, J.K., M.T., A.S.B.; Formal Analysis, M.R., Z.A.Z.; Investigation, Y.A.P., W.F.S.; Resources, M.R., Z.A.Z.; Data Curation, J.K., M.T.; Writing - Original Draft, Y.A.P., W.F.S.; Writing - Review & Editing, J.K., M.T.; Supervision, J.K., M.R.; Project Administration, J.K.; Funding Acquisition, J.K., M.R.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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# The ABC Analysis of Drug Use and Cost in Cardiology Outpatients - National Health Insurance

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***Abstract***

***Background****: Cardiovascular disease is one of the chronic diseases with an increasing prevalence from year to year. The results of Indonesia's basic health research (RISKESDAS 2018) showed that it was experienced by 2.9% of the population. Ensuring the continuity of drug availability is very important in pharmacy services for this patient group. The history of drug use is considered in the planning dan procurement of drug products.* ***Objective:*** *This study aims to analyze the drug use pattern and cost of drugs for cardiology outpatients in the National Health Insurance scheme (JKN).* ***Methods****: A retrospective observational study was conducted using the prescription of cardiology outpatients JKN for March-May 2021. The ABC method carried out the analysis of drug use patterns and cost.* ***Results****: From 2,986 prescriptions for cardiovascular disease, there were 94 types of drugs from 37 therapeutic classes. In the ABC analysis of drug use for class A, 68.82% contained seven types of drugs, class B, 20.55% contained nine types of drugs, and class C, 10.63% with 78 types of drugs. The results of ABC analysis for investment value (cost) of drugs class A 66.96% contained two types of drugs, class B 19.81% with four types of drugs, and class C 14.23% with 88 types of drugs.* ***Conclusion****: ABC analysis of the drug use pattern and cost/investment showed different patterns which had value to consider in procurement planning to maintain service continuity.*

***Keywords****: drug usage, cost, abc analysis, cardiovascular disease, JKN*

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## INTRODUCTION

The catastrophic disease has a high cost, high volume, and high risk, making many policymakers worry about increasing disease costs (Wasis & Mugeni, 2013). Cardiovascular disease is one of the catastrophic diseases that occupies the largest proportion of catastrophic financing in the JKN program (BPJS Kesehatan, 2020 type of drug is one of the elements that affect the cost of treating heart disease (Hussey, et al, 2002). The results of the 2016 Indonesia Health Financing Research showed that Social Health Insurance Administration Body (BPJS Kesehatan) support 61,13% for inpatient care and 62,03% for outpatient care, this medical service cost compared to other insurance financing sources. Another study shows that drug expenditure in Indonesia es 40-50% of operational health costs and increases annually. This critical issue encourages all related elements to calculate the actual health service cost (Suharmiati et al., 2019). The resources and management of health provide the continuity of the health financing subsystem (Arianto & Nantabah, 2020).

Cardiovascular disease is a chronic disease in which a cardiovascular drug procurement plan must be concerned to comply with the continuity of patient needs. The problems of drug shortage from e-catalogue drug procurement or inappropriate planning result in additional drug procurement regular prices, which exceed e-catalogue drug prices (Mendrofa & Suryawati, 2016). This condition disrupts the overall logistics management cycle starting from deficiencies in budgeting, procurement, management and storage of drugs (Walujo and Septria, 2017). Planning for drug needs in hospitals must be carried out using an accountable method to avoid drug shortage or drug excess. The correct method of drug procurement planning can improve hospital stock control (Kemenkes RI, 2019). ABC analysis is one of the analytical methods used to identify the types of drugs and costs by grouping drugs or costs into three groups. The three groups of ABC analysis are group A, which uses 70%, group B, which uses 20%, and group C, which 10% of the total drug or drug costs (Setiawati, 2020). ABC analysis attained a profile of effective and efficient drug use, which can be used as an overview for drug procurement planning in the following year. Therefore, this study was conducted to provide the drug use data and to analyze the pattern of drug use and costs in cardiology outpatient –JKN.

## MATERIALS AND METHODS

The study was designed as a retrospective observational study, analyzing the pattern of drug use and costs in cardiology outpatients-JKN using the ABC method. This study was carried out from drug the prescription of cardiology outpatient-JKN at the pharmacy department for outpatient services of Universitas Airlangga Teaching Hospital, Surabaya, Indonesia, from March to May 2021. The board has approved the methodology of this study of ethics of Universitas Airlangga Teaching Hospital number 151/KEP/2021.

### Data collecting procedure

This study's inclusion criteria were all prescriptions from cardiology outpatient-JKN at Universitas Airlangga Teaching Hospital, and there were no exclusion criteria. The data recorded in the data collection sheet include the type of drug, strength of the drug, route of administration, number of drugs and drug prices.

### Data analysis

Analysis of drug use and drug costs using ABC analysis, where group A with 70%, group B with 20% and group C with 10% of the total drug usage or drug cost.

## RESULTS AND DISCUSSION

### The pattern of drug use

This study obtained 2,986 prescribing and 37 therapeutic classes with 94 types of drugs used in cardiology outpatient-JKN. Total drug use based on the smallest unit (tablet, capsule) is 353,597. Ten (10) major therapeutic classes and drugs used are listed in Table 1. The therapeutic classification was based on the 2019 National Formulary, which can be accessed online through the e-Fornas website. According to the National Formulary, drugs are dispensed to JKN patients (Kemenkes RI, 2016).

The results showed that antihypertensive and heart failure drugs take the highest use of drugs in cardiology outpatient-JKN (Table 1). Beta Beta-blockers have a role in cardiovascular and non-cardiovascular treatment. Bisoprolol fumarate is the most widely used beta beta-blocker in this study. Bisoprolol is a selective beta-adrenoreceptor blocker; this drug selectively blocks beta-1 adrenoreceptors. Besides that, the antihypertensives frequently used are angiotensin receptor blockers (candesartan), calcium channel blockers (nifedipine ER), and angiotensin-converting enzyme inhibitors (lisinopril).

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**Table 1**. Top Ten Therapeutic Classes of Drug Use in Cardiology outpatient-JKN

| No | Drugs | Percentage (%) |
|---|---|---|
| 1 | Antihypertensive and heart failure drugs | |
| | - Bisoprolol fumarate 1.25 mg | 0.56 |
| | - Bisoprolol fumarate 2.5 mg | 18.69 |
| | - Bisoprolol fumarate 5 mg | 0.72 |
| | - Candesartan cilexetil 16 mg | 11.11 |
| | - Candesartan cilexetil 8 mg | 6.02 |
| | - Captopril 12.5 mg | 0.01 |
| | - Ramipril 2.5 mg | 0.86 |
| | - Ramipril 5 mg | 0.55 |
| 2 | Antihypertensive drugs | |
| | - Amlodipine besylate 10 mg | 0.88 |
| | - Amlodipine besylate 5 mg | 0.74 |
| | - Diltiazem HCl 100 mg SR | 0.26 |
| | - Diltiazem HCl 200 mg SR | 0.01 |
| | - Imidapril HCl 10 mg | 0.05 |
| | - Imidapril HCl 5 mg | 0.03 |
| | - Lisinopril dihydrate 10 mg | 0.58 |
| | - Lisinopril dihydrate 5 mg | 1.28 |
| | - Methyldopa 250 mg | 0.36 |
| | - Nifedipine 10 mg | 0.06 |
| | - Nifedipine 30 mg ER | 13.26 |
| | - Verapamil HCl 240 mg SR | 0.01 |
| 3 | Antianginal drugs | |
| | - Isosorbide dinitrate 5 mg | 3.65 |
| | - Nitroglycerine 2.5 mg ER | 6.12 |
| 4 | Antihyperlipidemic drugs | |
| | - Atorvastatin calcium trihydrate 20 mg | 2.67 |
| | - Fenofibrate 100 mg | 0.30 |
| | - Fenofibrate 300 mg | 0.11 |
| | - Gemfibrozil 300 mg | 0.05 |
| | - Simvastatin 10 mg | 0.01 |
| | - Simvastatin 20 mg | 6.60 |
| 5 | Platelet-aggregation Inhibitors drugs | |
| | - Acetylsalicylic acid 100 mg | 0.01 |
| | - Acetylsalicylic acid 80 mg | 7.02 |
| | - Clopidogrel bisulfate 75 mg | 0.84 |
| | - Ticagrelor 90 mg | 0.54 |
| 6 | Diuretic and heart failure drugs | |
| | - Furosemide 40 mg | 3.00 |
| | - Spironolactone 100 mg | 0.10 |
| | - Spironolactone 25 mg | 4.86 |
| 7 | Vitamin dan mineral | |
| | - Mecobalamin 500 mcg | 0.19 |
| | - Vitamin B complex | 1.19 |
| | - Vitamin B1 50 mg | 0.02 |
| | - Vitamin B12 50 mcg | 0.46 |
| | - Vitamin C 50 mg | 0.07 |
| 8 | Anticoagulant drugs | |
| | - Warfarin sodium 2 mg | 1.83 |
| 9 | Antihyperuricemic drugs | |
| | - Allopurinol 100 mg | 1.21 |
| | - Allopurinol 300 mg | 0.12 |
| 10 | Antacid and antiulcer agents | |
| | - Antacid 60 ml suspension | 0.01 |
| | - Antacid Doen | 0.06 |
| | - Lansoprazole 30 mg | 0.52 |
| | - Omeprazole 20 mg | 0.27 |
| | - Ranitidine HCl 150 mg | 0.12 |
| | - Sucralfate 500 mg/5 ml | 0.01 |

Note: SR = sustained release; ER = extended release

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**Table 2**. Grouping of Drug Usage with ABC Analysis in Cardiology Outpatient -JKN

| Group | Type of Drug | | Usage |
|---|---|---|---|
| | Number | % | % |
| A | 7 | 7.45 | 68.82 |
| B | 9 | 9.58 | 20.55 |
| C | 78 | 82.97 | 10.63 |
| Total | 94 | 100 | 100 |

**Table 3**. Type of drug and ABC Category Based on Drug Usage in Cardiology Outpatients-JKN

| No | Drugs | Group |
|---|---|---|
| 1 | Bisoprolol fumarate 2.5 mg | A |
| 2 | Nifedipine 30 mg ER | A |
| 3 | Candesartan cilexetil 16 mg | A |
| 4 | Acetylsalicylic acid 80 mg | A |
| 5 | Simvastatin 20 mg tab | A |
| 6 | Nitroglycerine 2.5 mg ER | A |
| 7 | Candesartan cilexetil 8 mg | A |
| 8 | Spironolactone 25 mg | B |
| 9 | Isosorbide dinitrate 5 mg tab | B |
| 10 | Furosemide 40 mg tab | B |
| 11 | Atorvastatin calcium trihydrate 20 mg | B |
| 12 | Warfarin sodium 2 mg | B |
| 13 | Ramipril 2.5 mg kaps | C |
| 14 | Clopidogrel 75 mg tab | C |
| 15 | Amlodipine besylate 5 mg | C |
| 16 | Bisoprolol fumarate 5 mg | C |
| 17 | Lisinopril dihydrate 10 mg | C |

Note: ER = extended release

In Indonesia, beta-blockers were used to treat heart failure, hypertension, and myocardial infarction. Bisoprolol and propranolol were frequently used in this therapy (Sari et al., 2020).

**ABC analysis**

***ABC analysis based on drug use***

The purpose of the ABC analysis of drug use is to determine the amount of drug use during a specific period which can be used as a reference for planning and procuring drugs to meet the patient's drug needs. Of the 94 drugs used in prescription during March – May 2021, they were grouped according to the amount of use value using the ABC system (70-20-10).

The results of the ABC analysis in Table 2 show that group A (68.82% of the total drug use) contained 7 (7.45%) drugs. Group B (20.55% of total use) had 9 (9.58%) drugs. Meanwhile, drugs that are included in group C (10.63% of total use) contained 78 (82.97%) drugs.

Table 3 contains a list of seven drugs from group A and five drugs from groups B and C. The seven drugs with the highest usage value or group A are bisoprolol 2.5 mg, nifedipine 30 mg ER, candesartan 16 mg, acetylsalicylic acid 80 mg, simvastatin 20 mg tab, nitroglycerine 2.5 mg ER, and candesartan 8 mg. Moreover, tablets of spironolactone 25 mg and isosorbide dinitrate 5 mg, Furosemide 40 mg tablets, atorvastatin 20 mg and warfarin 2 mg are group B drugs with a moderate therapeutic index. Meanwhile, group C or drugs with low usage values include ramipril 2.5 mg, clopidogrel 75 mg, and amlodipine 5 mg. Group A drugs are a group of drugs which frequently used and require firm supervision to prevent drug shortage. The availability of drugs for groups B and C should not be ruled out because the drugs from both groups are still needed by patients, which is reflected in their monthly use of these drugs supporting the patient's therapeutic needs, but there should not be an overstock of drugs in this group, especially group C. The number of Category C drugs related to therapy for cardiology cases was 32%, including ramipril 2.5 mg, clopidogrel 75 mg, amlodipine besylate 5 mg, bisoprolol fumarate 5 mg, lisinopril dihydrate 10 mg, diltiazem 100/200 mg, amiodarone 200 mg, verapamil 240 mg, simvastatin 10 mg.

***ABC analysis based on drug investment values***

Table 4 shows the results of the ABC analysis based on the investment value. The results showed that only 2 (2.13%) drugs were included in group A with an investment value of 66.96% of the total 3-month drug

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investment value. Group B contains 4 (4.25%) drugs with an investment value of 19.81%. Meanwhile, drugs belonging to group C consist of 88 (93.62%) drugs with an investment value of 14.23%.

Group A is a group of drugs consuming about 70% of the total investments. This drug requires tight control, low safety stock, centralized purchasing authority, and short lead times. Special care must be taken in monitoring and controlling because a high investment value can result in increased storage costs and losses in the event of drug damage.(Walujo and Septria, 2017; Sari et al., 2020).

Drug group B is a drug group that consumes approximately 20% of total investment and requires moderate control, moderate safety stock, decentralized purchasing authority, and moderate waiting time; however, administrative reporting must be strict and detailed because drugs in group B also provide a significant amount of investment in hospitals, albeit not as much as group A. This class of drugs must be properly stored.. In addition, stock checking for this group is only carried out based on changing needs. Although group C has a high number of drug items (88), this group consume investments of around 10% only. Regular control, administration and monitoring were done for this group, even though this class do not provide a high investment for the hospital. In addition, stock checks are less frequent than in group A and B drugs (Sari et al., 2020).

Table 5 shows only two types of drugs in group A: nifedipine 30 mg sustained-release tablets and nitroglycerin 2.5 mg sustained-release capsules. These two drugs are also included in group A in the list of A, B, and C drugs based on usage values (Table 3). Nifedipine is a potent peripheral arterial vasodilator that selectively inhibits the transmembrane influx of calcium ions into cardiac, smooth muscle and vascular, thereby reducing calcium influx, decreasing peripheral vascular resistance, and increasing cardiac output. Nifedipine has been developed with modified or delayed release mechanisms to improve therapeutic outcomes, as well as to improve patient compliance. Extended-release CCBs are recommended as first-line agents in treating ischemic heart disease. Another prospective indication is for patients at high risk of coronary disease and diabetes (El-Masry & El-Khodary, 2020).

ABC analysis of drug use and the value of drug investment show different patterns. Group A on the use of drugs contained seven drugs, while the investment value or costs contained 2 drugs. Group B contained nine drugs and four drugs for usage and investment value/cost, respectively. Nifedipine ER and nitroglycerin ER in the analysis based on usage were entered in groups A in the 2nd and 6th order. However, in the analysis based on the investment value, they were in the position of group A and only contained the two drugs, shifting the position of bisoprolol and the drug above nitroglycerin ER in Table 3. This is because the price of ER nifedipine is eight times higher than bisoprolol (Rp. 3,986.00 vs. Rp. 495.00), and the cost of ER nitroglycerin is more than three times higher than bisoprolol (Rp. 1,640,00 vs Rp. 495.00) causing a shift in the position of nifedipine ER and nitroglycerin ER in investment analysis. This difference needs to be considered in planning the supply of drugs; there are drugs with high investment value along with another group A drugs to maintain the availability of drugs and continuity of pharmacy services. Category A drugs in this study were antihypertensive, antiplatelet, and vasodilator drugs, in line with the existing diagnoses, 41.5% hypertension and 26.8% coronary artery disease. The percentage of ABC category drugs from several studies showed different values. Category A drugs for tertiary hospitals in India and university hospitals in Turkey were 21.22 and 12%, respectively (Mathew, et al, 2016); Yigit, 2017). For planning, analysis from each institution is required.

ABC analysis considers the amount of use only and has not evaluated the drugs that must be available because they are live-saving/vital drugs even though they are used in small quantities. For JKN patients, further analysis with ABC-VED (Vital-Essential-Desirable) combination is required, in addition to conformity analysis with the national formulary.

**Table 4**. Grouping of Investment Values with ABC Analysis in Cardiology Outpatient -JKN

| Group | Type of Drug | | Investment Values |
|---|---|---|---|
| | Number | % | % |
| A | 2 | 2.13 | 66.96 |
| B | 4 | 4.25 | 19.81 |
| C | 88 | 93.62 | 14.23 |
| **Total** | **94** | **100** | **100** |

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**Table 5**. Type of drug and ABC Category Based on Investment Values in Cardiology Outpatients-JKN

| No | Drugs | Group |
|---|---|---|
| 1 | Nifedipine 30 mg ER | A |
| 2 | Nitroglycerine 2.5 mg ER | A |
| 3 | Bisoprolol fumarate 2.5 mg | B |
| 4 | Ticagrelor 90 mg | B |
| 5 | Candesartan cilexetil 16 mg | B |
| 6 | Candesartan cilexetil 8 mg | B |
| 7 | Diltiazem HCl 100 mg SR | C |
| 8 | Atorvastatin calcium trihydrate 20 mg | C |
| 9 | Warfarin sodium 2 mg | C |
| 10 | Bisoprolol fumarate 1.25 mg | C |
| 11 | Simvastatin 20 mg | C |
| 12 | Acetylsalicylic acid 100 mg | C |

Note: ER = extended release; SR = sustained release

## CONCLUSION

The use of drugs for cardiology outpatient-National Health Insurance in Airlangga University Teaching Hospital includes 37 therapeutic classes with 94 types of drugs. Analysis of drug usage patterns and costs shows different drug patterns that need to be considered in drug supply planning. Based on the value of use and investment, drugs included in groups A, B and C were 7.45% vs. 2.13; 9.58% vs. 4.25%; 82.97% vs 93.62%, respectively. The results of this study are expected to be used as references in drug procurement planning by considering aspects of use and investment value.

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## AUTHOR CONTRIBUTIONS

Conceptualization, B.S., M.Y.; Methodology, B.S., M.Y., M.D.S.; Software, M.D.S., S.F.A.; Validation, B.S., M.Y.; Formal Analysis, M.D.S., S.F.A., D.D.; Investigation, M.D.S., S.F.A.; Resources, M.D.S., S.F.A.; Data Curation, M.D.S., S.F.A.; Writing - Original Draft, B.S., M.D.S., D.D.; Writing - Review & Editing, B.S., M.D.S., D.D.; Supervision, B.S., M.Y.; Project Administration, M.D.S., S.F.A.; Funding Acquisition, B.S.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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# Analysis of Antibiotic Use in COVID-19 Patients at a Hospital in Sidoarjo

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***Abstract***

***Background****: Antibiotics are given to COVID-19 patients to treat bacterial co-infections, but excessive and inappropriate antibiotic use can increase antibiotic resistance.* ***Objective:*** *The study aims to analyze the antibiotic use and bacterial susceptibility in COVID-19 patients at a hospital in Sidoarjo - East Java.* ***Method:*** *The research design used was a prospective cross-sectional study using data collection of sputum culture, bacterial susceptibility, medical records, and Pharmacy antibiotics usage from August to October 2021. The Research sample is moderate-to-severe COVID-19 patients.* ***Result:*** *The results showed that in August-October 2021, 32 patients met the study sample category, where the most were men (56.25%), age range 46-55 years (31.25%), and outcome recovered as much as 81.25%. The culture results showed that only 1 (one) person out of 32 samples had bacterial growth. The only bacteria that grew in the sputum culture was Enterobacter aerogenes, susceptible to meropenem but resistant to levofloxacin. The highest quantity of antibiotics in this study was levofloxacin 71,16; meropenem 32.25; moxifloxacin 21.84 DDD/ 100 bed days.* ***Conclusions:*** *Most of the cases of COVID-19 occurred in men; the age range was 46-55 years, and the outcome recovered was more than those who died. Bacterial co-infection occurred in 3.12% of patients who received meropenem and levofloxacin during their hospitalization. The largest quantity of antibiotics used was levofloxacin, meropenem, and moxifloxacin. The combination of meropenem and levofloxacin should be re-evaluated because levofloxacin is resistant but still susceptible to meropenem.*

***Keywords****: COVID-19, antibiotics resistant, sputum culture, ATC/DDD methodology*

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## INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) infection causing current health problems. The World Health Organization (WHO) officially declared the infection as Corona Virus Disease 2019 (COVID-19)in February 2020 (Hu et al., 2021, Wang et al., 2020). COVID-19 is the third pandemic in the 21st century caused by the Corona Virus, with the highest mortality rate (Khan et al., 2021).

The SARS-CoV-2 infection causes various symptoms, such as anosmia, hyposmia, dysgeusia, shortness of breath, dry cough, fever, and weakness. The majority of symptoms occur in the respiratory tract, and radiology examination showed bilateral pneumonia. Other manifestations that occur in COVID-19 include complications in the heart, gastrointestinal, liver, peripheral, and central nerves (Wang et al., 2020; Tsatsakis et al., 2020). The symptoms generally occur in COVID-19 patients, but it should be noted that some people have no symptoms (Setiadi et al., 2020).

SARS-CoV-2 infection was first identified in Wuhan China. The virus has spread not only in China but also to other countries around the world. The cases of COVID-19 continue to increase every day, WHO data until April 11, 2021, shows this pandemic has spread to more than 200 countries around the world with the number of confirmed cases having been infected by this virus as many as 135,057,587 cases and 2,919,932 deaths. The highest cases occurred in America, with 58,025,495 cases and 1,405,254 deaths. In Asia, the number of confirmed cases was 16,177,826 cases and 228,385 deaths.1,437,283 confirmed cases were reported on March 17, 2021, in Indonesia, making Indonesia the country with the highest number of cases in Southeast Asia. Based on a map of the distribution of cases per province in Indonesia, East Java province, as of March 14, 2021, was in the top fourth rank after DKI Jakarta, West Java, and Central Java, with the number of confirmed cases in East Java of 134,595 cases. At the district/ city level, Sidoarjo district ranks second with the highest number of cases in East Java after Surabaya, with 10,481 cases (WHO, 2021; Satgas COVID-19, 2021).

Curative steps for infected patients need to be carried out optimally, although until now, there is no recommendation of drugs and vaccines that have been approved as prophylaxis or treatment of COVID-19 because they are still in the clinical trial stage (Neldi et al., 2020). Therapy is based on pre-existing therapies taking into account the pathogenesis of COVID-19 (Cao et al., 2020). The therapy is expected to reduce the Case Fatality Rate (CFR) in Indonesia. Until now, there is no COVID-19 drug that has received a distribution permit from the National Agency of Drug and Food Control of Indonesia (BPOM). BPOM is still issuing approval for emergency use authorization (EUA) which is expected to increase the cure rate and reduce mortality in COVID-19 patients.

Bacterial co-infection is common in viral infections and a significant reason for morbidity and mortality, but much information is still unknown regarding the incidence of co-infection in COVID-19 patients (Mahmoudi, 2020). One therapy often given to COVID-19 patients is antibiotics to treat co-infection or secondary bacterial infections. In a systematic review of 24 studies involving 3,338 COVID-19 patients who were evaluated for acute bacterial infection, 71.9% of patients received antibiotic treatment, although 3.5% were identified as having co-infection and 14.3% of secondary bacterial infections (Langford et al., 2020). Other studies related to the use of antibiotics in COVID-19 patients were performed on 340 patients, where 43 patients (12.46%) had secondary bacterial infection with Klebsiellasp (25.59%), Methicillin-sensitive staphylococcus aureus (MSSA) (20.93%), Escherichia coli (16.28%), methicillin-resistant staphylococcus aureus (MRSA)(13.95%), Enterobacter sp (11.63%), Streptococcus pneumonia (2.32%) and Pseudomonas aeruginosa (9.30%). The result of the study showed that Enterobacteriaceaeisolates from COVID-19 had the highest resistance to cotrimoxazole (74%), piperacillin (67.5%), ceftazidime (47.5%), and cefepime (42.5%). Data shows that excessive use of antibiotics in the COVID-19 pandemic can pose a risk of multi-resistant bacteria (PDPI et al., 2020). All isolates showed susceptibility to amikacin (100%), and the rate of resistance to oxacillin, erythromycin, and clindamycin was more than 90% (Mahmoudi, 2020). Other risks that can occur when excessive and irrational use of antibiotics are drug toxicity, super-infection risk, prolonged length of stay, and increased cost of treatment (Alldredge et al., 2013; Suda et al., 2014).

Antibiotic resistance is a major problem in human development and a threat to health, affecting the ability to fight several infections (WHO, 2018). One of which is the implementation of an antimicrobial resistance

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control program (PPRA) in hospitals must be aware of antimicrobial resistance control. Evaluation of the implementation of PPRA is carried out through an evaluation of antibiotic use (quantity and quality audit) and monitoring of the emergence and spread of resistant microbes through surveillance of resistant microbes (Kemenkes, 2015).

Standardized methods are required to assess and compare antibiotic use, which is done quantitatively using the anatomical therapeutic chemical (ATC) method for classifying drugs and the defined daily dose (DDD) method for measuring drug use based on WHO recommendations. This method can be used to compare the number of drugs between hospitals and countries (WHO, 2016). A retrospective study in Spain comparing antibiotic use before and during the COVID-19 pandemic using the ATC DDD method resulted in an increase in antibiotic consumption, especially in the intensive care unit (ICU). The rise in the consumption of these antibiotics was in the daptomycin, carbapenem, linezolid, ceftaroline, novel cephalosporin/ beta-lactamase inhibitor, or triazole groups during April – May 2020. Several antibiotics that experienced a significant increase were ceftriaxone, carbapenem, daptomycin, azithromycin, and linezolid (Grau et al., 2021).

Given the lack of information related to antibiotic use in COVID-19 patients, further research is needed to analyze the antibiotic use, the bacterial isolate in sputum samples, and antibiotic susceptibility in moderate-severe COVID-19 patients. This research was conducted at a Hospital in Sidoarjo regency, a national COVID-19 referral hospital that has treated patients with indications of COVID-19 with or without comorbidities.

## MATERIAL AND METHODS

The research method used was a prospective cross-sectional study. Data were taken from data collection of sputum culture, bacterial susceptibility, medical records, and antibiotics usage from the Pharmacy from August – October 2021.

The population in this research were COVID-19 patients who were hospitalized in the COVID-19 isolation ward at Sidoarjo Regional General Hospital from August to October 2021. All patients were laboratory-confirmed positive by using RT-PCR. The sample is the population that meets the inclusion and exclusion criteria. The inclusion criteria in this study were all adults (≥18 years old) and moderate-to-severe COVID-19 patients. The exclusion criteria were patients referred to another hospital and pregnant and lactating patients. Sampling was carried out using the consecutive purposive sampling method, where all COVID-19 patients who came and met research criteria according to the research objectives were included in the study until the required number of subjects was met for data analysis. The sample size in this study is the entire sample that meets the criteria, with a minimum sample of 30 (Gay et al., 2012).

Data analysis was carried out descriptively in the form of an overview of bacterial isolates in sputum samples, antibiotic susceptibility, the profile of COVID-19 patients based on therapeutic outcomes during hospitalization, and measuring the antibiotic use using the ATC DDD/ 100 patients bed day method. This research has received ethical approval from the Health Research Ethics Commission of the hospital in the Sidoarjo district with no 893.3/017/438.6.7/2021.

## RESULTS

About 32 COVID-19 patients met the inclusion criteria of the study. The sample was taken with a sputum specimen during hospitalization in the isolation ward for culture examination. The demographic features of moderate-severe COVID-19 patients according to Table 1.

**Table 1.** Demographic Features

| Variable | total | Percentage (%) |
|---|---|---|
| Gender | | |
| Male | 18 | 56.25 |
| Female | 14 | 43.75 |
| Age | | |
| 18-25 | 2 | 6.25 |
| 26-35 | 3 | 9.375 |
| 36-45 | 6 | 18.75 |
| 46-55 | 10 | 31.25 |
| 56-65 | 6 | 18.75 |
| ≥ 65 | 4 | 12.5 |

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**Table 2.** Patient Profile Based on Therapeutic Outcome

| Therapeutic Outcome | Total | Percentage (%) |
|---|---|---|
| Recovered | 26 | 81,25 |
| Died | 6 | 18,75 |
| Total | 32 | 100 |

**Table 3.** Culture and Isolated Bacteria

| Sputum Culture of Bacteria | Total | Percentage (%) |
|---|---|---|
| Negative | 31 | 96.875 |
| Positive | 1 | 3.125 |
| Total | 32 | 100 |
| Bacteria Isolated | | |
| *Enterobacteraerogenes* | 1 | 100 |
| Total | 1 | 100 |

**Table 4.** Antibacterial Susceptibility

| | | *Enterobacteraerogenes* |
|---|---|---|
| ATC Code | Antibiotic | N (1) |
| J01CA01 | Ampicillin | R |
| J01CR01 | Ampicillin/Sulbactam | R |
| J01CR05 | Piperacillin/Tazobactam | S |
| J01DB04 | Cefazolin | R |
| J01DD02 | Ceftazidime | S |
| J01DD04 | Ceftriaxone | S |
| J01DE01 | Cefepime | S |
| J01DF01 | Aztreonam | S |
| J01DH03 | Ertapenem | S |
| J01DH02 | Meropenem | S |
| J01GB06 | Amikacin | S |
| J01GB03 | Gentamicin | S |
| J01MA02 | Ciprofloxacin | S |
| J01AA12 | Tigecycline | S |
| J01XE01 | Nitrofurantoin | R |
| J01EE01 | Trimethoprim +Sulfamethoxazole | S |
| J01MA12 | Levofloxacin | R |

Noted: N = total isolate, R=Resistant, S=Susceptible

**Patient profile based on therapeutic outcome**

The sample was categorized based on the therapeutic outcome (recovered or died) during hospitalization. According to Table 2. the number of patients recovered was 81.25%and the rest died (18.75%).

**Culture and isolation of bacteria**

Sputum cultures were obtained from 32 COVID-19 patients. The sputum specimen was then cultured on blood agar and MacConkey agar plates and then incubated for 18-24 hours at $37^0$C. One positive culture (3.125%) was obtained, and then the isolated bacteria was identified using the standard microbiological method. The bacteria isolated from sputum culture was *Enterobacteraerogenes*, as shown in Table 3.

**Data analysis**

Analysis of drug use and drug costs using ABC analysis, where group A with 70%, group B with 20% and group C with 10% of the total drug usage or drug cost.

**Antibacterial susceptibility**

Isolated bacteria were tested for antibacterial susceptibility based on CSLI M100-S24.[20]The results of antibacterial susceptibility as shown in Table 4.

**Suitability of antibiotic use based on antibacterial susceptibility results**

The patient with positive sputum culture *Enterobacteraerogenes* had received the combination of levofloxacin 750 mg/24h and meropenem 1 gr/8h antibiotics during hospitalization. Based on the susceptibility results, as shown in table 5, levofloxacin is not suitable, but the use of meropenem is suitable, so

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the use combination of meropenem and levofloxacin antibiotics could be overcoming the bacterial infection.

**Analysis of antibiotic use based on ATC DDD/100 patient bed days method**

During the period August - October 2021, it was found that all samples received antibiotics. The antibiotics were then categorized based on the ATC code recommended by WHO so that 11 groups of antibiotics were obtained. The calculation results of DDD/100-bed days areshown in Table 6.

## DISCUSSION

The demographic data showed that the number of males (56.25%) more than females (43.75%), which is according to a study in China that 52% of COVID-19 patients were male as well as a study from Europe which also showed that 72% were male (Jin et al., 2020; Raimondi et al., 2021). The more significant number of males indicates that males experience more severe illness and higher mortality than females (Jin et al., 2020). The difference in the number and severity of males being greater than females in COVID-19 cases could be due to comorbidities and several high-risk behaviours such as smoking by men (Cheng et al., 2021). In addition, the expression of Angiotensin-Converting Enzyme 2 (ACE-2) in males is more significant than in females because of different sex hormones, so males are more easily infected with COVID-19 and have poorer clinical outcomes. However, the relationship between ACE_2 and COVID-19 mortality still needs to be reviewed because other studies have shown that the presence of ACE-2 can protect against organ damage (Biswas et al., 2021).

The highest incidence of COVID-19 in this study occurred at the age of 46-55 years (31.25%), where this age was included in the early elderly category (Kemenkes, 2020). This data is slightly different from other research in that the median incidence of COVID-19 is mainly at 62 years (late elderly) (Jin et al., 2020). COVID-19 could occur at any age, but those aged ≥ 50 years have a significantly higher risk of death (Biswas et al., 2021). Elderly patients face a greater risk of worsening because there will be changes in the body's physiology and the presence of comorbidities. The elderly have natural immunity that decreases with aging, affecting the adaptive and innate immune systems to control viral infection, making them more susceptible to infection (Leng et al., 2010). The elderly are also more vulnerable to unwanted effects from drugs used to treat comorbidities (Lavan et al., 2016). Moderate-severe COVID-19 patients who recovered in this study were 81.25 %, slightly different from another study in that the number of recovered patients in all categories was 96.50 % (Jin et al., 2020).

**Table 5.** Antibiotics Suitability Based on Antibacterial Susceptibility Results

| No | Antibiotic | Antibacterial Susceptibility Result | Suitability |
|---|---|---|---|
| 1 | Levofloxacin | Resistant | Not suitable |
| 2 | Meropenem | Susceptible | Suitable |

**Table 6.** DDD/100 Bed Days COVID-19 patients

| No | Antibiotic | ATC code | Route of Administration | DDD WHO Standard | DDD/ 100 Bed Days |
|---|---|---|---|---|---|
| 1 | Levofloxacin | J01MA12 | Parenteral | 0.5 | 71.1604 |
| 2 | Meropenem | J01DH02 | Parenteral | 3 | 32.2526 |
| 3 | Moxifloxacin | J01MA15 | Parenteral | 0.4 | 21.8430 |
| 4 | Ceftazidime | J01DD02 | Parenteral | 4 | 8.9590 |
| 5 | CefoperazoneSulbactam | J01DD62 | Parenteral | 4 | 3.0717 |
| 6 | Azithromycin | J01FA10 | Parenteral | 0.5 | 2.9010 |
| 7 | Ceftriaxone | J01DD04 | Parenteral | 2 | 0.1706 |
| 8 | Amoxicillin and Beta-Lactamase Inhibitor | J01CR02 | Parenteral | 3 | 0.1138 |
| 9 | Metronidazole | J01XD01 | Parenteral | 1.5 | 0.1138 |
| 10 | Azithromycin | J01FA10 | Oral | 0.3 | 4.5506 |
| 11 | Trimethoprim-sulfamethoxazole | J01EE01 | Oral | NA | - |

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Radiology finds that COVID-19 patients are often characterized by the presence of pneumonia that is difficult to distinguish between bacterial or viral causes, so clinicians provide antibiotic therapy. In this study, all subjects received antibiotic therapy. In COVID-19 patients, antibiotic therapy is generally not recommended unless bacterial pneumonia is proven. Giving antibiotics to COVID-19 patients must be careful because they have the potential for excessive use in the COVID-19 pandemic era, which can become a global threat to the increase in the incidence of multi-resistant bacteria (Alldredge et al., 2013).In patients with the possibility of bacterial pneumonia, antibiotic therapy with first-line empirical antibiotics can be given, daily re-evaluation is carried out, and if there is no evidence of bacterial infection, then de-escalation or discontinuation of antibiotics is carried out.55 Therapeutic guidelines for CAP (Community-Acquired Pneumonia), which is not severe, the recommended therapy regimen is a combination of beta-lactam + macrolide or a single respiratory fluoroquinolone. In contrast, for patients in the severe category, beta-lactam + macrolide/ beta-lactam + respiratory fluoroquinolone can be used (Joshua et al., 2019). Fluoroquinolones, carbapenems, protein synthetase inhibitors, cephalosporins, beta-lactams, metronidazole, and cotrimoxazole were the antibiotics used by the patients in this study. The group that was mostly used was fluoroquinolones, followed by carbapenems. The fluoroquinolones used in COVID-19 patients in this study were moxifloxacin or levofloxacin, while the carbapenem group used meropenem. Fluoroquinolones are a group of antibiotics that have a broad spectrum. Fluoroquinolones have the potential as antivirals to suppress the replication of multiple positive-sense viruses and single-stranded RNA viruses such as dengue virus, zika virus, rhinovirus, and hepatitis C virus, presumably through the mechanism of interference with viral entry and viral helicase inhibition. A study was conducted to determine the antiviral potential of fluoroquinolones (enoxine, ciprofloxacin, levofloxacin, and moxifloxacin) and yielded data that these fluoroquinolones are not suitable antivirals for COVID-19 (Scroggs et al., 2020). Patients in this study also received meropenem which is generally combined with fluoroquinolone. The administration of fluoroquinolones and meropenem is by the therapeutic guidelines, but the administration is only for bacterial pneumonia. Evaluation of the antibiotics utilization quantitatively was then carried out using the method recommended by WHO, namely ATC DDD/100 bed days. The DDD/100 bed days of antibiotic treatment calculation in moderate-to-severe COVID-19 patients from August to October 2021 obtained a total result of 145.1365 DDD/100 bed days. Levofloxacin is an antibiotic with the highest value, 71.16 DDD/bed days, followed by meropenem 32.25 and moxifloxacin at 21.84 DDD/100 bed days. This study is similar to a study that analyzed the antibiotics utilization with the ATC DDD/ 100 bed days method in the early COVID-19 pandemic where there was the use of beta-lactam antibiotics (ceftriaxone, carbapenems, cephalosporins/beta-lactamase inhibitors) and azithromycin in a hospital (Gay et al., 2012). Another study stated that the most antibiotics given to COVID-19 patients were azithromycin, levofloxacin, and ceftriaxone, with the value of DDD/bed days respectively 48.12; 44.01; and 21.13 DDD/ 100 bed days (Putra et al., 2021). Sputum cultures of COVID-19 patients were carried out on research samples and observed bacteria growing to determine the possibility of bacterial co-infection in patients. From the total sample of the study, 32 specimens of sputum were obtained, but only one specimen (3.125%) experienced bacterial growth. The bacteria that grew in the sputum specimen was Enterobacteraerogenes. Enterobacteraerogenes is commonly found in the human gastrointestinal tract and does not cause health problems in healthy humans (Bains et al., 2020). This bacteria was found in COVID-19 patients in another research (Catano-correa et al., 2021). Based on the percentage of samples containing bacteria in the sputum of COVID-19 patients, the bacterial co-infection in this study was 3.125%, where the results were not different from existing meta-analysis studies, namely the incidence of co-infection in COVID-19 patients was 3.5% (PDPI et al 2020). Other bacteria were not found in the other 31 sputum because the infection was caused by the covid-19 virus without bacterial co-infection. The covid-19 virus is the cause of pneumonia experienced by patients. Administration of antibiotics for COVID-19 patients requires further review because COVID-19 infection is caused by a virus, not bacteria, unless the patient has a bacterial co-infection. This low proportion of bacterial co-infections in COVID-19 patients indicates that antibiotics are not appropriate for COVID-19 patients unless it is proven that the patient has bacterial co-infection.

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In this study, isolate that were positive experienced bacterial growth and then tested for antibiotic susceptibility. The results of these tests revealed various types of data susceptibility and resistance. Piperazine/tazobactam, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, and trimethoprim + sulfamethoxazole were all effective against the isolate. At the same time, the isolates showed resistance to the antibiotics ampicillin, ampicillin-sulbactam, cefazolin, nitrofurantoin, and levofloxacin. The research samples were taken from the sputum, and the isolates experienced the growth of Enterobacteraerogenes bacteria in this study, receiving antibiotic levofloxacin 750 mg and meropenem 1 gr. Based on the antibiotic sensitivity test data, the use of meropenem was appropriate because the isolates still showed susceptibility to the antibiotic, but not for the benefit of levofloxacin because the isolates showed resistance to the antibiotic. Although the results of the susceptibility test of levofloxacin are resistant bacteria, the bacterial co-infection in this patient can still be overcome by meropenem, where meropenem is a broad-spectrum antibiotic group and is still susceptible based on the results of the bacterial sensitivity test.

Based on the culture and bacterial susceptibility test results, which were only obtained from 1 (one) research sample, other studies related to bacterial co-infection in COVID-19 patients are needed as comparison material.

## CONCLUSION

Most of the cases of COVID-19 occurred in men; the age range was 46-55 years, and the outcome recovered was more than those who died. Bacterial co-infection occurred in 3.12% of patients, where they received meropenem and levofloxacin during their hospitalization. The most significant quantity of antibiotics used was levofloxacin, meropenem, and moxifloxacin. The combination of meropenem and levofloxacin should be re-evaluated because levofloxacin is resistant but still susceptible to meropenem.

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## AUTHOR CONTRIBUTIONS

Conceptualization, R.Y., F.H.; Methodology, R.Y., F.H.; Validation, W.K. F.H.; Formal Analysis, W.K. F.H.; Investigation, W.K.; Resources, W.K.; Data Curation, W.K.; Writing - Original Draft, W.K. F.H.; Writing - Review & Editing, W.K. F.H., R.Y.; Supervision, W.K..; Project Administration, F.H., R.Y.; Funding Acquisition, R.Y.

## CONFLICT OF INTEREST

The authors report no conflict of interest in this study.

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