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FOREWORD

Alhamdulillah, praised to Allah, Journal *Qanun Medika: Fakultas Kedokteran Universitas Muhammadiyah Surabaya* vol 04 no 02 has been published. It consists of 15 articles including 2 literature reviews, 1 case report and 12 research articles in the medical field. We would like to thanks our reviewers and editorial board members who helped us in this publication. In order to be internationalized, we only published articles written in English since July 2019. We hope that these articles can be read widely both by domestic and foreign readers.

Thank you,
Yelvi Levani, MD.,M.Sc
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Literature Review

Quality of life of cervical cancer patients

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Abstract

Cervical cancer is known as the highest cause of death after breast cancer. It is gynecological cancer contributing to the top cause of death in women. Sexual and reproductive issues can reduce the quality of life of women with cervical cancer. WHO defines the quality of life as an individual's perception in the cultural context and value system in which the individual experiences and is related to their goals, expectations, standards, and affairs. It gives the concept of an individual's physical health, psychological conditions, one's beliefs, social relationships, and one's involvement with something meaningful in their environment. This article will explain the definition, symptoms, and staging of cervical cancer, the definition of the quality of life, the meaning of excellence for the cervical cancer patient, and the facts that affect the quality of life.

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INTRODUCTION

Cervical cancer, based on the data from the Global Burden Cancer or the International Agency for Research on Cancer (IARC), is known as the highest cause of death after breast cancer. This cancer is gynecological cancer contributing to the most top cause of death in women,with 84% of new cases worldwide (Memon & El-Turki, 2018). In the developed countries, programs are in place, which enables females to be vaccinated against HPV and women to get screened regularly. Screening allows pre-cancerous lesions to be identified at stages when they can quickly be treated. Early treatment prevents up to 80% of cervical cancers in these countries (World Health Organization, 2019).

Cervical cancer in Indonesia is the most common cancer with an incidence rate of 23.4 per 100,000 population, with 13.9 deaths per 100,000 people (Kemenkes RI, 2015). This condition makes cervical cancer called the number one killer disease in Indonesia. The deaths associated with the majority of cervical cancer stages (80%) are invasive, advanced, and even terminal stages at the time of diagnosis (Nindrea, 2017). The highest prevalence of people with cervical cancer in Indonesia in 2013 was 0.8% or an estimated 98,692 patients. The province of Kepulauan Riau, North Maluku, and Daerah Istimewa Yogyakarta had the highest prevalence of cervical cancer in Indonesia. In 2013, the North Sumatra Province ranked ninth with a prevalence of 0.7% or an estimated 4,694 patients (Kemenkes RI, 2013).

Cervical cancer will cause its problems for women who experience it because it is associated with changes in the female reproductive organs, which are considered a significant part of women (Wijaya, 2010). A cervical cancer diagnosis is an emotional trauma for women. The impacts of cervical cancer are decreased self-esteem on body image, impaired relationships with partners, sexual, and reproductive issues that can reduce the quality of life of women with cervical cancer (Samadi, 2010).

WHO defines the quality of life as an individual's perception in the cultural context and value system in which the individual experiences and is related to their goals, expectations, standards, and affairs (Yulianti et al., 2015). It gives the concept of an individual's physical health, psychological conditions, one's beliefs, social relationships, and one's involvement with something meaningful in their environment.

This article aims to look at the meaning of the quality of life of people with cervical cancer.

LITERATURE REVIEW

CERVICAL CANCER

Definition of Cervical Cancer

Cervical cancer is cancer that grows on the cells of the cervix caused by infection with Human Papilloma Virus (HPV) and is transmitted directly through skin contact during sexual intercourse in patients who have infected with the HPV virus. Human Papilloma Virus (HPV) is a virus that attacks the skin and mucous membranes of humans and animals (Arum, 2015).

Cancer is an uncontrolled growth of body cells forming new cells that urges healthy cells and causes problems in the organs where cancer grows (American Cancer Society, 2017). The cervix is a cylindrical lower part of the uterus that connected to the vagina. At the top meet, the corpus of the uterus is called isthmus or internal os, and the lower border of the cervix related to the vagina is called external os. The cervix is anatomically divided into endocervix and ectocervix, which is coated by two different glands. The columnar epithelium paints the endocervix while the ectocervix covered by

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squamous epithelium, and the two organs meet at the squamocolumnar junction (SCJ) (Bermudez et al., 2015).

Risk Factors for Cervical Cancer

Factors that cause cervical cancer include:

1) *Genetic factors*
Genetic abnormalities play a role in carcinogenesis and cervical tumor aggressiveness around 32-34%. Families with a history of cervical cancer can increase their risk two to three times higher than those without a family history of cervical cancer (Kessler, 2017).

2) *Sexual behavior*
Sexual behavior is related to the age of first sexual intercourse and the number of sexual partners or partners with many sex partners. They are related to the possibility of cervix exposed to higher and longer carcinogenic factors. Women who first have sexual intercourse at the age of <20 years old have more risk than women who have sexual intercourse >20 years old (Kessler, 2017).

3) *Reproductive factors*
Higher parity, early age at first birth, and the number of vaginal deliveries cause chronic trauma to the cervix during childbirth to be a factor in cervical cancer (Roura et al., 2016; Kessler, 2017). A woman who experiences her first pregnancy age of 17 is almost twice as likely to develop cervical cancer than a woman who waits to get pregnant until the age of 25 (Kessler, 2017).

4) *Smoking habits*
Female smokers with high duration and intensity show a twofold increase in the risk of grade 3 cervical intraepithelial neoplasia (NIS 3)/ carcinoma in situ (KIS). Women who smoke are twice as likely to get cervical cancer compared to nonsmokers. Cancer-causing chemicals and tobacco byproducts in cigarettes have been found in the cervical mucosa of female smokers, and these substances damage the DNA of cervical cells (Kessler, 2017).

5) *The use of long-term oral contraceptives*
The use of oral contraceptives for more than five years has a risk of cervical cancer, but the risk returns to normal ten years after oral contraception is stopped (Roura et al., 2016; Kessler, 2017).

6) *History of Sexually Transmitted Diseases (STDs)*
Chlamydia and herpes simplex infections cause chronic inflammation and ulcerative micro-changes in the cervical epithelium that play a role in cancer initiation and progression (Roura et al., 2016).

7) *Chronic immunosuppression*
Women with advanced Human Immunodeficiency Virus (HIV) infection have a high risk of cervical cancer because the development of pre-cancerous lesions becomes invasive cancer more quickly (Kessler, 2017). HIV is a virus that damages the immune system. The immune system is essential in destroying cancer cells and slowing growth and spread. For women with HIV, cervical precancers lesion develop invasive cancer faster than usual. Having HIV makes a woman's immune system less able to fight both HPV infection and diseases at an early stage (Ngabo et al., 2016).

8) *Dietary factors*
Diets high in calories and sugar, sugary drinks, and processed meat are associated with weight gain, leading to obesity, which risks increasing carcinogenesis. A healthy diet with high vegetable food intake (fruits, vegetables, legumes, and whole grains), low consumption of processed red meat, moderate consumption of sweet foods, and avoidance of high salt intake are associated with lower cancer risk and improve cancer prognosis for the better in patients who have to diagnose with cancer (Norat et al., 2015).

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9) *Factors of poverty*
The poverty factor is associated with low income and limited access to health care cannot be screened for cervical cancer precursors or treated for cervical cancer (Kessler, 2017).

Symptoms of Cervical Cancer

Several signs found in patients with advanced cervical cancer, according to Arum (2015), namely:

1) Abnormal leucorrhoea
Recurrent leucorrhoea occurs, which does not heal even though it has been treated. The leucorrhoea is usually smelly, itchy, and hot because it has a secondary infection, meaning that the fluid coming out of the pre-cancerous lesion or cancer is added to contamination by germs, bacteria/parasites, fungi, and even HPV virus infections.

2) Bleeding from the vagina
At an advanced stage, cervical cancer symptoms not only cause vaginal discharge but also bleeding from the vagina. This bleeding occurs outside the menstrual period. It can occur after intercourse, too much force during bowel movements, or after menopause.

3) Pain in the reproductive organs
People affected by cervical cancer will also often experience pain in the area around the vagina. Apart from the vaginal area, the pain will usually also felt in the lower abdomen, thighs, and hip joints during menstruation, bowel movements, and intercourse. If cervical cancer has spread to the pelvis, the patient will suffer complaints of back pain, obstacles in urination, and kidney enlargement.

Staging Cervical Cancer

The diagnosis of the staging of cervical cancer is crucial for proper treatment. The stage of cervical cancer is divided into five types. According to Cancer Research UK (2019), the types of cervical cancer given as follows:

1) Normal
This is also called "Carcinoma In Situ (CIS)," which means that some cervical cells change the changes. However, abnormal cells are located and contained in the cervix's surface layer and are still in place. Carcinoma in situ is not cancer, but in some women, the change will develop into cancer after a few years.

2) Stage 1
It is characterized by cancer cells that only exist in the cervix, and the size of the disorder is less than 3 mm. This stadium means that cancer only exists in the uterus. Usually divided into two stadiums, namely:
a) Stage 1A: minimal growth can only see with a microscope. Stage 1A1 means that cancer has grown less than 3 millimeters (mm) into cervical tissue, and is less than 7 mm wide. Stage 1A2 means cancer has grown between 3 and 5 mm into the cervical tissue but is still less than 7 mm wide.
b) Stage 1B: the cancer area begins to spread, but it is still only in the cervical tissue and has not spread. It can be seen without a microscope but can not see. In stage 1B1, the cancer is no larger than 4 cm. In 1B2 glass, the tumor is more significant than 4 cm.

3) Stage 2
Cancer has begun to spread in the cervix into the surrounding tissue. Nevertheless, it not yet grown into the muscles or ligaments that line the pelvis (pelvic wall) or the bottom of the vagina. This stage is divided into two sub stage, namely:
a) Stage 2A: cancer has spread to the upper part of the vagina

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JURNAL KEDOKTERAN FKUM SURABAYA
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b) Stage 2B: cancer spreads to the tissue around the cervix.

4) Stage 3
Cancer has spread outside the uterus but is still in the pelvic cavity and has not entered the bladder or rectum. However, lymph consumption can already contain cancer cells. Cancer at this stage is high-grade cancer, and the symptoms are getting worse. The stage 3 is divided into two, namely:
a) Stage 3A: this stage prohibits cancer cells from spreading to the lower third of the vagina but not to the pelvic wall.
b) Stage 3B: the cancer cells have spread to the pelvic wall and can even deny because of its enlarged size. This blockage can cause the kidneys to stop working.

5) Stage 4
Cancer has spread to the bladder, rectum, or others. Stage 4 is also divided into two, namely 4A and 4B.
a) Stage 4A: cancer has spread to the bladder, and the rectum also covers lymph.
b) Stage 4B: cancer has spread beyond the pelvis and includes lymph nodes besides the pelvis such as liver, stomach, lungs, digestive tract, bones.

QUALITY OF LIFE

Definition Quality Of Life

Quality of life is a subjective perception of satisfaction or happiness in life and an essential domain for individuals (Ikatan Dokter Anak Indonesia, 2017). According to WHO in Astuti et al. (2015), if the patient's quality of life decreases, the patient will feel physically, psychologically, socially, and spiritually uncomfortable. The patient also cannot utilize his life optimally for the happiness of himself and others.
There are four fields (areas) used to measure the quality of life, namely physical health, psychological health, social relations, and the environment (Salim et al., 2016). In detail, the areas of quality of life assessment include:

a) The domain of physical health, related matters, include daily activities, dependence on medical materials or medical help, energy and fatigue, mobility, pain and discomfort, sleep and rest, and work capacity
b) Psychological domain, related things such as body image and appearance, negative and positive feelings, spirituality / personal beliefs, thoughts, learning, memory, and concentration
c) The social domain, related matters such as personal relationships, social relations, and social support, and sexual activity
d) Environmental area, about financial resources, freedom, physical security and safety, health and social care (accessibility and quality), home environment, opportunities to obtain information and learn new skills, opportunities for recreation or leisure, physical environment (pollution, noise, traffic, climate), and transportation

Result Facts Affects Quality of Life

Factors that can affect the quality of life include:

1) *Physical Factor*
According to research conducted by (Khalid et al., 2016), physical factors such as functional disabilities can affect the quality of life of individuals because these individuals cannot undergo their daily activities independently. Anxiety and pain sometimes make the patient unable to work as usual and impedes daily activities or routines (Utami et al., 2014) The frequent pain that interferes with daily activities, lack of energy in activities, lack of satisfactory sleep quality, and lack of ability to work can also affect the quality of life (Rose et al., 2020).

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1) *Psychological Factors*
Psychological factors, such as depression can reduce a person's quality of life (Lin et al., 2015). In addition to depression, dementia (Khalid et al., 2016), reduced ability to concentrate, feeling dissatisfied with yourself due to illness and frequent negative feelings such as loneliness, despair, and anxiety also become the factors that can affect the quality of life (Astuti et al., 2015).

2) *Clinical Factors*
Clinical factors that can affect the quality of life of individuals including the side effects in medicine (Lin et al., 2015), disease severity, and complications (Khalid et al., 2016), drug use, and patient compliance (Yaghoubi et al., 2012).

3) *Socio-Economic Factors*
According to Khalid et al. (2016), marital status, family status, and health services can affect the quality of life of individuals. Huang et al. (2017) states the factor related to QOL of cervical cancer survivors was household income.

4) *Family Support*
The family is an aspect in making decisions concerning where treatment should be given and by whom. Family support can make the family able to improve health and adaptation in life so that it will affect the quality of life where information support included in the family health care function of family members. This information support can provide in the form of giving advice, direction, and essential information needed. In dealing with these conditions, family support is necessary. Family support is to help or support the family in the form of attention, appreciation, and love in a family. The backing that is owned by someone can prevent the development of problems due to pressure faced, according to Prodono et al. (2009).

THE MEANING OF QUALITY FOR CERVICAL CANCER PATIENTS

Made et al., (2017) stated that cervical cancer patients treated in the Gynecology Cempaka Room at Sanglah Hospital Denpasar are mostly advanced-stage cases. Physically, most cases of cervical cancer complain of feeling a lack of energy/weakness at an intermediate level even though only a small proportion complain of severe nausea. Weakened physical conditions make it difficult for sufferers to meet with the family. This condition also influences by feelings of pain, where most sufferers complain of moderate-severe pain. Declining physical health also influenced by complaints due to the side effects of the drug. Most of them experience physical activity obstruction due to claims of drug side effects. Physical conditions like this also have an impact on the time spent in bed.

Research of Sabulei & Maree (2019) showed that women treated for cervical cancer get financial difficulties that were rampant, especially during the treatment phase. Insomnia and urinary frequency were the most cumbersome problems and remained so even after treatment. Cervical cancer and its treatment had a negative influence on QoL in all domains of the lives of these women.

Research of Endarti et al. (2015) showed that the most frequently reported problems were pain/ discomfort, followed by anxiety/depression. Cervical cancer significantly affects patient's health-related Quality of Life (HRQoL). Research of Thapa et al. (2018) in China stated symptoms that mostly experienced were insomnia, constipation, financial difficulties, and menopausal symptoms.

A study in Ethiopia suggests that HRQoL patients with cervical cancer in Ethiopian were low with mean global health status/ QoL score of 48.3 ± 23.77 and EQ-5D index of 0.77. Physical functioning, emotional functioning, pain, and symptom experience significantly

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http://journal.um-surabaya.ac.id/index.php/qanunmedika

affects the global health status/ QoL (Araya et al., 2020).

The study of de Arruda et al. (2020) identified that after treatment for locally advanced cervical cancer, patients improved in most quality of life aspects. However, worsening observed in sexual enjoyment, peripheral neuropathy, and menopausal symptoms. Efforts should be made to prevent and treat these long-term effects of locally advanced cervical cancer treatment, which could improve patients' quality of life.

Study of dos Santos et al. (2019) indicated that health-related quality of life in women with cervical cancer using domains of HRQoL of the women treated for cervical cancer, showed a better score observed in the areas of physical and social/ family well-being. Most domains show better scores in those with a current occupation, the duration after the diagnosis and treatment, and among those who had undergone a hysterectomy.

Research on cervical cancer survivors', regarding the quality of life and sexual function, states QoL and sexual function of cervical cancer survivors' were lower than the general population. Treatment-related complications and sexual dysfunction significantly affected patients' QoL. Having health insurance was associated with better QoL. Sexual function was adversely affected by radiotherapy and radical hysterectomy. Sexuality is an essential concern of cervical cancer survivors (Zhou et al., 2016). Due to traditional culture, cervical cancer survivors in China usually avoid discussing issues of sexuality with physicians. A model gynecological advice clinic has shown positive outcomes in British cancer patients who face sexuality issues. Therefore, a similar strategy could be adopted to improve cervical cancer survivors' sexual function and QoL in China.

CONCLUSION

Cervical cancer has an impact on the quality of life, either physical, psychological, social, or financial. Physical changes can occur, including on the reproductive organs. Sexuality, insomnia, and urinary frequency are the most complicated problems. The symptoms that remain even after treatment are pain or discomfort, followed by anxiety or depression, reduced loss of appetite, fatigue, and financial difficulties.

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Literatur Review

The level of effectiveness use of Quinoline Drugs in COVID-19

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Abstract

Chloroquine is the first line of medicine in the treatment of malaria. Besides being antimalaria, the chloroquine also can be used as the anti-inflammation in the medicine of arthritis rheumatoid arthritis and lupus erythematosus discoid. Hydroxychloroquine sulfate is 4-aminoquinolin with hydroxylated chloroquine analog, having the same pharmacokinetic as chloroquine which is given orally in hydroxychloroquine sulfate form, processed by gastro-intestinal absorption and very faster kidney elimination. The effectiveness of chloroquine and hydroxychloroquine towards COVID-19 in the in vitro experiment showed it could inhibit the duplication of the SARS-CoV-2 virus. The chloroquine function is to stop COVID-19 infection with (EC50) 1,13 µM and (CC50) larger than 100 µM. Meanwhile, the hydroxychloroquine function is to inhibit the attachment and entry of the virus into the host's cell by enzymatic activation which is the lysosome acidification disorder and antigen presentation as the result of pH increase. Based on the clinical study, the 10 of 12 patients who have lopinavir/ritonavir therapy by virology, the chloroquine group showed RT-PCR negative on day 7, 10, and 14 in compare to lopinavir/ritonavir that showed RT-PCR negative on day 14. On the 9th day, 60% of the patients of chloroquine group showed the CT scan of Lungs image normal instead of the lopinavir/ritonavir at 25%. In the day 14 based on the CT test result, the pulmonary improvement increased twice rather than chloroquine group (Rate Ratio 2.21). Meanwhile, the result of the study on the hydroxychloroquine and *azithromycin* combination use showed a decrease in viral load of 83% and 93% in tests with negative results in the day 7 and 8. It proved that the chloroquine role showed the result of the medicine has a significant effect by cleaning the virus or other clinical matters. The purpose of this literature review is to know the effectiveness quinoline class of drugs which is chloroquine and hydroxychloroquine in COVID-19 disease.

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INTRODUCTION

The COVID-19 pandemic started in China last December 2019 located in Wuhan. In an attempt to stop the spread, in January 2020, this virus had spread widely in Asia country, and the first identification case of the virus's spread was in Europe. Initially, this disease named a 2019 novel coronavirus (2019-nCoV). On February 11 2020, WHO officially named this disease as Coronavirus Disease 2019 (COVID-19) that is caused by a novel coronavirus. Moreover, Coronavirus is a new disease for the people that became a big threat in a large society (WHO, 2020).

There is some accepted medicine by FDA as the COVID-19 therapy, such as ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine, and two antiviruses broad-spectrum medicine redeliver and favipiravir to the clinical symptoms of COVID-19 by in vitro. Among the seven medicines it was found a high concentrate test from three nucleoside analog which is ribavirin (EC50 = 109,50Mm), penciclovir (EC50 = 95,96 µM), favipiravir (EC50 = 61,88 µM), nafamostat (EC50 = 22,50 µM), nitazoxanide micromolar (EC50 = 2,12µM), remdesivir micromolar (EC50 = 0,77µM), and chloroquine (EC90 = 6,90µM).

The chloroquine and 4-aminoquinoline hydroxychloroquine are the quinoline family that has the same molecule. The hydroxychloroquine is different from chloroquine because of the hydroxyl group existence at the end of the substituent side-chain N-ethyl βhidroksilasi (Devaux et al., 2020). Chloroquine itself is distributed all over the body including lungs after given orally. The chloroquine and hydroxychloroquine function are known to block the virus infection by increasing the pH endosome which is needed for the fusion of virus/cell also disturbing the glycosylation cellular receptor of SARS-CoV-2 (Wang et al., 2020).

The use of chloroquine itself must be based on the rules and not for self-medication. Generally, the phosphate chloroquine and hydroxychloroquine are commercialized as the antimalaria medicine that is used in an autoimmune disease like lupus and rheumatoid arthritis. There is a special consideration in using chloroquine and hydroxychloroquine that is considered safe and mild side effects so can consider the use of chloroquine and hydroxychloroquine dose margin therapy to avoid chloroquine poisoning and not cause cardiovascular disorder complication that can be life-threatening (Touret & Lamballerie, 2020).

METHODS

The method used is by collecting and analyzing the articles related to the effectiveness of quinoline drugs which is chloroquine and hydroxychloroquine in COVID-19 disease. The articles were obtained by using an electronic database searching from Google Scholar, PubMed, and Elsevier using the keywords Effectiveness, Quinoline, Chloroquine, Hydroxychloroquine, and COVID-19. The reviewed articles are from the year 2010-2020 that discussing COVID-19, in full-text format, that specifically discusses the effectiveness of quinoline drugs which is chloroquine and hydroxychloroquine in COVID-19 disease.

COVID-19 DISEASE

Coronavirus is a beta coronavirus that has RNA single strand genome and helical capsid with envelopes that consisted of lipid bilayer diameter 60-100nm. The sequence analysis of the COVID-19 genome showed that it has substantial similarity as Coronavirus like SARS, which mostly infected bats, which later mutate and infect humans (Frater et al., 2020). Coronavirus is one of the diseases that the spread of infection is very fast through the respiratory tract, so that can affect the syndrome of symptoms acute breathing because of SARS-

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CoV-2 (Rothan & Byrareddy, 2020). The incubation period is 2-14 days until the patient showed symptoms, including fever (99%), cough (50%), and breathing difficulty (33%) as the most common complaint. Mostly, about 80% of infected people have mild to moderate symptoms, and the rest are quite severe (WHO, 2020). The COVID-19 patient with respiratory tract medical records tend to have more severe clinical manifestations (Yang et al., 2020). The Centers for Disease Control and Prevention (CDC) announced some other risk factors that can cause the COVID-19 infection starting from the highest to lowest, having close contact to COVID-19 patient, being in the same environment but no contact, and arriving from infected country history (CDC, 2020).

Based on the data and study cases result, it showed that age, gender, active smoker, and having a comorbid disease such as diabetes mellitus and hypertension are the risk factors of SARS-CoV-2 infection. The elderly are more susceptible to SARS-CoV-2 since they have higher possibility frequency related to the comorbid disease. Males have a higher prevalence rather than females, and it was predicted to associate with a higher tendency of an active smoker. The SARS-CoV-2 infection will be related to their target cell through angiotensin-converting enzyme 2 (ACE2), which is expressed by epistle pulmonary cells, kidney, and blood vessels. As to the smoker, hypertension, and diabetes mellitus, it was predicted that there is an increased receptor expression of ACE 2. (Cai, 2020).

Pathogenesis COVID-19

ACE2 is a protein-membrane type I which facilitates the virus attachment to a cellular receptor, initiation infection, and angiotensin-converting enzyme 2 (ACE2) that is expressed to lungs, heart, kidney, and intestine so it can be identified as a functional cellular receptor of SARS-CoV-2. (Jin et al., 2020). Based on his analysis, Zhao showed that angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2. In the lungs of normal people, ACE2 is expressed to the epistle cell of alveolar I and II. The relation of SARS-CoV-2 to ACE2 causes an expression increase of ACE2 that can cause alveolar cell damage. The damage of alveolar cells can trigger some systematical reactions and even mortality (Sun et al., 2020). The first clinical description showed by the infected people through the COVID-19 pandemic is respiratory symptoms. The spread of COVID-19 from humans to humans becomes the main transmission source so the spread becomes more aggressive. The transmission of SARS-CoV-2 happened through droplet that comes out from the cough or sneezing (Han & Yang, 2020).

Chloroquine

Chloroquine is prophylaxis drugs formed amine acidotropic of quinine and hydroxychloroquine that mostly used as malaria drugs before. Based on in vitro study, the chloroquine function is to block COVID-19 infection in low micromolar concentration by the effective concentration of half the maximum (EC50) 1,13 μM and 50% cytotoxic concentration (CC50) larger than 100 μM (Gao et al., 2020). From its pharmacokinetics travel of chloroquine, it is given orally in a tablet in the form of phosphoric acid. The chloroquine in metabolism becomes the active metabolite and desethyl chloroquine through cytochrome liver enzymes P450 (CYP) 2C8 and CYP3A4. The mechanism of chloroquine is distributed widely in most organ systems including eyes, heart, liver, lungs, and last secreted through the kidney. In COVID-19 medication based on the expert's study, they suggested to use phosphate chloroquine tablet by dosage 500mg per oral twice or in 10 days (Barlow et al., 2020) and from pharmacodynamics mechanism, the

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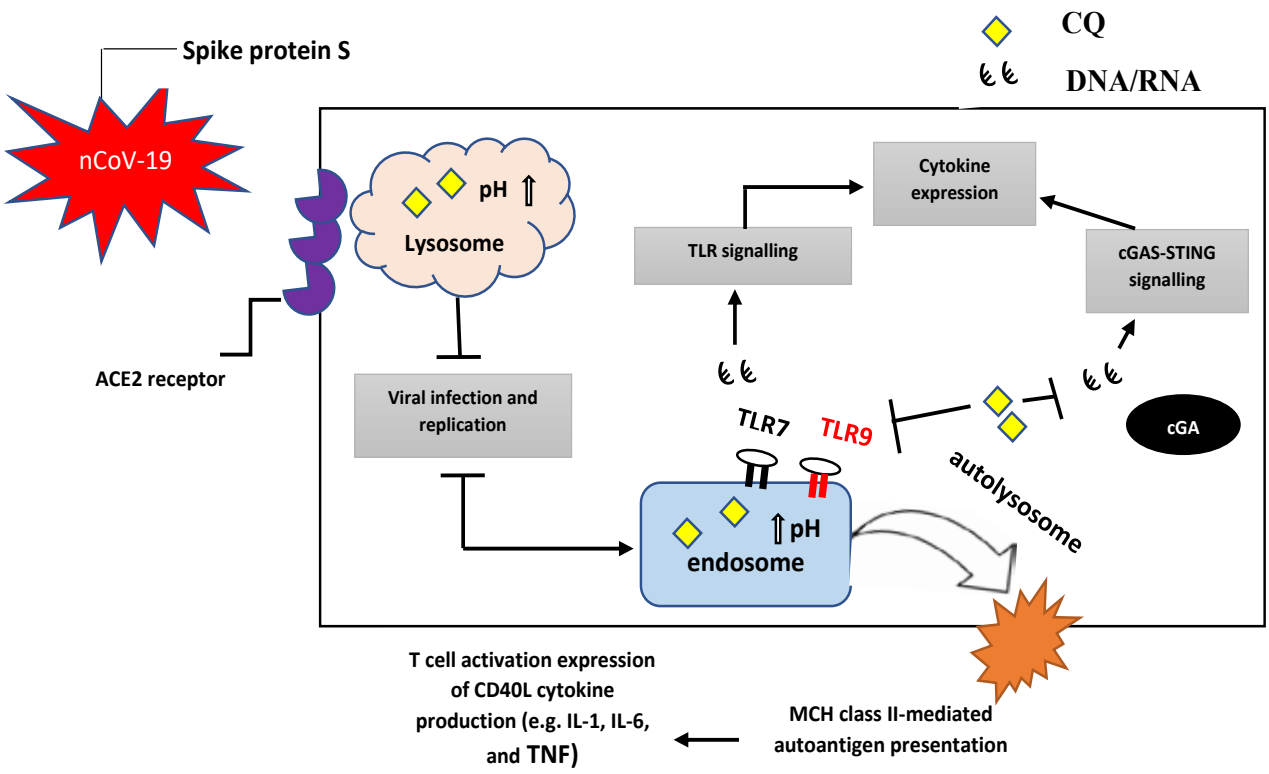

Figure 1. Mechanism of chloroquine towards COVID-19

chloroquine drugs work to change glycosylation by increasing pH intracellular vacuole level and change the protein degradation track through acid hydrolase in lysosomes, macromolecule synthesis in the endosome, and protein modification of post-translation in Golgi body so there is an antigen macrophage activation in reaching antirheumatic response that can disturb a process of antigen (Sahraei et al., 2020)

The base chloroquine works by penetrating cells that acid concentrated so cause an increase of pH endosome. This condition can be the potential therapeutic strategy to virus infection that can inhibit the production of some cytokine, chemokine, or mediator that exaggerated in contributing to the virus infection severity (Vincent et al., 2005). Moreover, the chloroquine can set the immune system by influencing cell signal and cytokine proinflammation production (Zhang et al., 2020). In orderly, the chloroquine non-protonated entering cells by concentrating on acid organelle becomes protonated, the low pH like endosome, Golgi vesicles, and lysosome. The chloroquine can work effectively in influencing virus based on their amount by using endosome as the mediator of virus duplication (Al-Bari, 2017). The indication of chloroquine use for prophylaxis malaria treatment is sensitive to chloroquine (P. falciparum, P. Ovalle, P. Vivax, and P. malaria) and as extraintestinal amebiasis treatment. Meanwhile, the Covid-19 treatment is given to people who indicate an Acute Respiratory Distress Syndrome (ARDS) symptoms such as asphyxiated and breathing difficulty. Besides, the chloroquine contraindicated cannot be used to the patient with retinal disorder or insight except for acute malaria treatment, 4-*aminoquinoline* hypersensitivity, and QT interval extension. The drug administration of chloroquine can be given to prophylaxis for malaria orally with the dose 500mg two weeks before, during, and 8 weeks after exposure to endemic areas. In amebiasis, the drugs given

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orally in the dose 21mg/kg during 3 weeks and for severe malaria treatment can be given parenterally or subcutaneous (Goel & Gerriets, 2019).

The use of chloroquine suggested for an adult is consist of 600 mg base chloroquine (6 tablets CQ 100 mg) followed by 300 mg after 12 hours on the day 1, then 300 mg (2x a day) on the 2-5 day, and 500 mg (2x a day) (Cortegiani et al., 2020). The side effects of chloroquine consumers are nausea, vomit, stomachache, diarrhea, cough, shortness of breath, and rash or itchy (Huang et al., 2020). The monitoring that can be conducted when using chloroquine is including initial electrocardiogram (ECG), electrolyte, kidney function, and liver test.

Some complications might happen in using chloroquine that can make QT extension with insufficient or kidney failure, increasing the insulin level that can cause severe hypoglycemia, caused hemolysis to the patient with glucose-6-phosphate dehydrogenase (G6PD), even interacting with other drugs can risk the QT extension even after have stopped the drugs in a long time about 30-60 days (FDA, 2020).

Hydroxychloroquine

Hydroxychloroquine sulfate is 4-aminoquinolin as chloroquine analog which is hydroxylated. Generally, this drug mostly the same as chloroquine which is used as the antimalaria drug. This drug role is inhibiting plasmodial polymerase.

Some analysis result mentioned that hydroxychloroquine can heal varied condition such as diabetes mellitus, dyslipidemia, coagulopathy, infectious diseases, malignancy, and some autoimmune disease, arthritis rheumatoid, and Systemic Lupus Erythematosus (SLE) (Ponticelli & Moroni, 2017).

Hydroxychloroquine has the same pharmacokinetic with chloroquine which is given orally in hydroxychloroquine sulfate form, by the absorption gastrointestinal process, and faster kidney elimination (Devaux et al., 2020). Besides being antimalaria, hydroxychloroquine also has some mechanism effects of antiinflammation such as lysosome

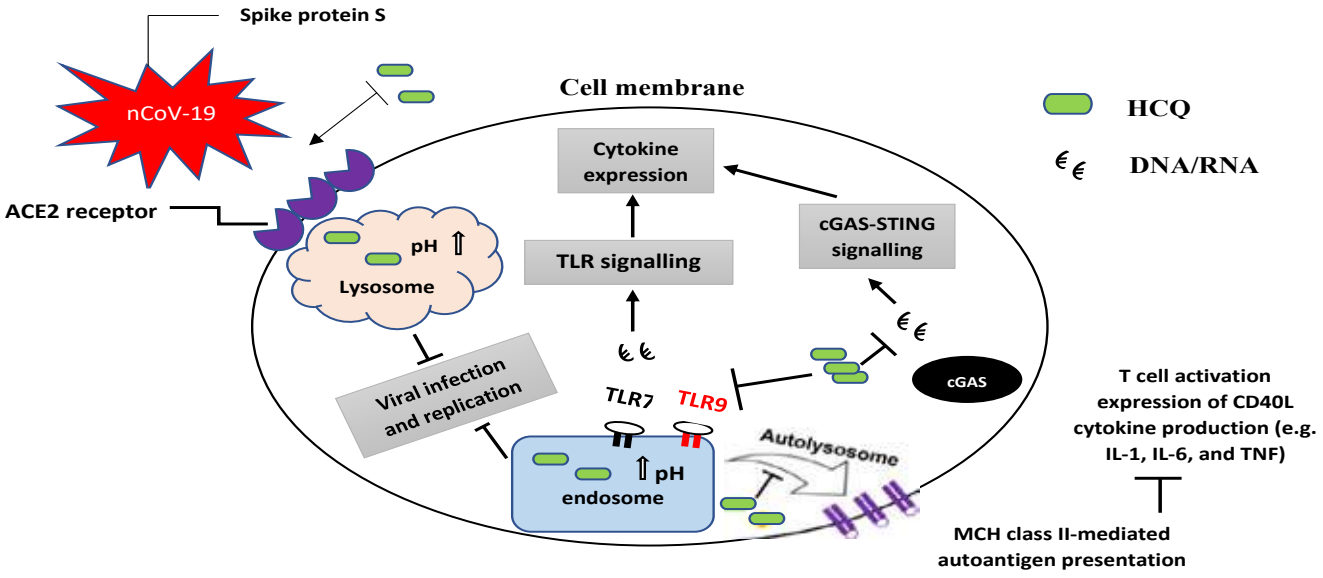

Figure 2. Mechanism of hydroxychloroquine towards COVID-19

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acidification disorder and antigen presentation, A2 phospholipase absorption inhibitor, absorption and blocker of UV skin reaction, binding and stabilizing DNA, TLR signal inhibitor, calcium T cell receptors inhibitor, and decreasing cytokine production which using macrophage media such as interleukin IL-1 and IL-6. The interaction between TLR and cell receptors can cause hydroxychloroquine more effective in some flow signal locations as the response of inflammation (Sinha & Balayla 2020).

Furthermore, it also has an anti-virus effect mechanism effect that is hydroxychloroquine inhibits and attaches the virus entry to the host's cell. This proofed to decrease Phosphatidylinositol Binding Clathrin Assembly Protein (PICALM) so it can arrange the cellular endocytosis rate which is mediated by Clathrin-Mediated Endocytosis (CME) as the mediator that has a role when SARS-CoV-2 entering human's cell. Next, S protein from Coronavirus will undergo proteolytic that based on endosomal protease acid cellular, for example, cathepsin or Transmembrane Serine Protease 2 (TMPRSS2).

In the process of cleavage resulting membrane endosome virus fusion that can be inhibited by pH increase. The process is the enzymatic activation phase from both cathepsin and TMPRSS2 which have an effect in virus attachment, entering the host cell, and block the virus inside endocytic vesicles. The mechanical effect of another antivirus is the inhibition of maturation and the spread of a new virus particle. In this phase, the hydroxychloroquine acts as endosomal alkalization, inhibits or prevents endosome-lysosome membrane fusion that leads to recycling viral membrane receptor process, the virus release, and virus genome inside the cytosol of SARS-CoV-2 virus (Quiros Roldan et al., 2020).

The indication of hydroxychloroquine use is to treat autoimmune diseases such as Systematic Lupus Erythematosus (SLE) and rheumatoid arthritis and also use to prevent and medicine of malaria. Meanwhile, the Covid-19 treatment is given to people who indicate an Acute Respiratory Distress Syndrome (ARDS) symptoms such as asphyxiated and breathing difficulty (Meyerowitz et al., 2020). The contraindication of hydroxychloroquine use is on retina disorder or retinopathy, cardiomyopathy, Long QT Syndrome, psoriasis arthritis, porphyria, and neuropathy (Pastick et al. 2020).

The dosage given is 3x200mg hydroxychloroquine for 10 days but the treatment can be varied from 5 to 20 days based on the clinical level especially in respiratory disorder (Cortegiani et al., 2020). The side effect of hydroxychloroquine can appear as gastrointestinal symptoms such as nausea, vomit, unusual shortness of breath, cardiotoxicity that leads to QT abnormalities (Hashem et al., 2020). The possible monitoring in using hydroxychloroquine is hematology parameter (RBC, WBC, and thrombosis amount), measuring electrolyte serum, blood glucose, and liver and kidney function (Singh et al., 2020).

The Effectiveness of chloroquine and hydroxychloroquine in COVID-19

Recently, the specific anti-virus treatment is not found yet for COVID-19. However, the effective supportive treatment still being an urgent need as the temporary treatment to decrease a mild or medium clinical symptoms up to 5-10% and potentially life-threatening weight of COVID-19 is chloroquine/hydroxychloroquine, remdesivir, and lopinavir-ritonavir (Şimşek Yavuz & Ünal, 2020).

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supportive treatment still being an urgent need as the temporary treatment to decrease mild or moderate clinical symptoms up to 5-10% and potentially life-threatening weight of COVID-19 is chloroquine/hydroxychloroquine, remdesivir, and lopinavir-ritonavir (Şimşek Yavuz & Ünal, 2020).

Chloroquine is anti-malaria drugs which are mostly used as the immunomodulatory effect meanwhile hydroxychloroquine based on the past study mentioned that can treat SARS plague. It is proofed that medicine has an activity of anti-SARS-CoV by in vitro. But until recent days, there is no clinical proof that shows if hydroxychloroquine can treat SARS-CoV-2 (Yao et al., 2020). On the other way, there are some studies that stated that chloroquine has cytotoxic concentration 50% (CC50). It proofed that the chloroquine activity is very selective in fighting virus duplication from the host's cell (Liu et al., 2020).

By in vitro, chloroquine and hydroxychloroquine 4-aminoquinolin are a low base that increases the endosomal pH of the host's intracellular organelle that can inhibit autophagosome-lysosome fusion and non-activated the enzyme needed by the virus to duplicate. The role of COVID-19 can affect the glocalization of angiotensin-converting enzyme-2 (A2) so it comes to the result that can inhibit SARS-CoV-2. More than 100 patients showed that phosphate chloroquine is more effective in inhibiting exacerbation (Ferner & Aronson, 2020).

Besides, the chloroquine has a function in modulating the immune system activity that synergistically increases the antivirus effect by in vitro. It proofed that by in vitro, the chloroquine is very effective in controlling COVID-19 infection. There is some proof that has been conducted in China for the COVID-19 patient who consumes chloroquine orally that is distributed to lungs and all over the body and have evidence of clinical achievement (EC50) 6,90 μM in Vero E6 that is gained by patient's plasm of rheumatoid arthritis in the dose of drugs 500 mg (Wang et al., 2020).

Based on the National Health Commission of the PRC study result, the chloroquine is used back as the emergency therapy of COVID-19 treatment. There are 82 patients who are screened COVID-19 test and 22 of them showed positive criteria of SARS-CoV-2 after conducted the RT-PCR test. the confirmed 22 patients are mixed into two first groups 10 people treated by using chloroquine 500 mg per oral two twice a day, including the 3 patients of severe level and 7 patients of moderate level. Next, in the second group of 12 people treated by lopinavir/ ritonavir 400/100 mg per day for 10 days, including 5 patients of severe level and 7 patients of moderate level. The level of effectiveness in the chloroquine group showed that 1 people is negative after 2 days of treatment. It shows that chloroquine experienced earlier development

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Table 1. Clinical study of chloroquine in COVID-19 patient

82 patient (n=22)	Patient	Clinical Status	Treatment	Duration	Percentage
1.	10 patients	3 severe 7 moderate	Chloroquine 2x500 mg/day per oral	14 days	60 % pulmonary improvement
2.	12 patients	5 severe 7 moderate	Lopinavir/ritonavir 400/100 mg/day	10 days	25 % pulmonary improvement

Table 2. Clinical study of hydroxychloroquine in COVID-19 patient

	Researcher	Patient	Group Control	Treatment	Duration	Improvement Percentage
1.	Gautret et al	20 patients	16 patients	Hydroxychloroquine 200 mg/8 hour or azithromycin 500 mg/first day and 250 mg/2-5 days after	10 days	17%
2.	Gautret et al	80 patients with Inclusive criteria and exclusive 69 out of 75	NO	Hydroxychloroquine 200 mg/8 hour and azithromycin 500 mg/first day and 250 mg/2-5 days after	10 days	93%
3.	Chen et al	30 patients	15 patients	Hydroxychloroquine 200 mg/12 hour	7 days	86,7%
4.	Chen et al	62 patients	31 patients	Hydroxychloroquine 200 mg/12 hour	5 days	80,6%
5.	Molina et al	11 patients	NO	Hydroxychloroquine 200 mg/8 hour and azithromycin 500 mg/first day and 250 mg/2-5 days after	10 days	57,1%

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from lopinavir/ritonavir treatment, and having an improvement on day 13 that accumulated as a patient with chloroquine treatment showed a negative result. Based on the clinical study, the 10 of 12 patients who have lopinavir/ritonavir therapy by virology, the chloroquine group showed RT-PCR negative on day 7, 10, and 14 in compare to lopinavir/ritonavir that showed RT-PCR negative on day 14. On the 9th day, 60% of the patients of chloroquine group showed the CT scan of Lungs image normal instead of the lopinavir/ritonavir at 25%. In the day 14 based on the CT test result, the pulmonary improvement increased twice rather than chloroquine group (Rate Ratio 2.21). This proved that the chloroquine treatment has better effectiveness in COVID-19 in stopping the virus duplication and repairing the pulmonary function faster (Huang et al., 2020).

The given clinical data of hydroxychloroquine in the first study is from Philippe Gautret by using 20 participants that consumed hydroxychloroquine and 6 of them also consumed azithromycin. About 16 patients are controlled under the short analytical observation for 6 days showed results in the intervention and control groups. 17% of patients did not show any symptoms and only 22% of them has pneumonia. The second study, even though it showed a bigger result but did not have a control group. Besides, inclusive and exclusive criteria showed that 69 of 75 patients or about 92% had a similar clinical result with the patient that had no COVID-19 treatment. The combination of hydroxychloroquine and azithromycin showed a decrease in the viral load of 83% and 93% tested with a negative result in day 7 and 8. Jun Chen's study of 30 patients found that there is no significant difference in the nasopharynx virus test on day 7 with local treatment standards. The second test of Zhaowei Chen in 62 patients of hydroxychloroquine treatment showed a clinical recovery in a short period of time such as temperature and cough. The last study in 11 patients showed the SARS-CoV-2 persistence in nasopharynx swab from 8 out of 10 patients that received hydroxychloroquine treatment (Taccone et al., 2020).

The Gautret et al. study result stated that China experts used chloroquine and hydroxychloroquine in giving COVID-19 patient clinical treatment. It is because the role of chloroquine and hydroxychloroquine towards the growth of SARS-CoV-2 is by in vitro, showed a result that chloroquine has a significant effect in inhibiting the virus duplication or even the clinical matters. Moreover, in chloroquine treatment mentioned, the experts suggested that the patients diagnosed as pneumonia COVID-19 in the degree of mild, moderate, and severe without contradiction to chloroquine can be treated with the dose 2x500 mg for 10 days (Gautret et al., 2020).

The chloroquine and hydroxychloroquine have as similarity in increasing intracellular acid organelles pH such as endosome or lysosome fusion mechanism membrane. The chloroquine function is to inhibit the SARS-CoV-2 entry so there will no protein improvement by glycosylation receptor process ACE2. Meanwhile, the hydroxychloroquine is effective in inhibiting the entry of SARS-CoV-2 and post-event after SARS-CoV-2 entry. Both of them can be differentiated by the cytotoxic level that showed hydroxychloroquine lower than 40% than chloroquine (Liu et al., 2020). There are some studies about the difference in effectiveness between chloroquine and hydroxychloroquine. Based on Yao et al., he tested the antivirus in the Vero cell line that is infected by SARS-CoV-2. The result is hydroxychloroquine is more effective in disturbing virus duplication instead of chloroquine that is given after the infection with EC50 in 48 hours each 0,72μM and 5,47μM for hydroxychloroquine and chloroquine after given prophylaxis EC50 in 48 hours that is 5,85 μM dan 18,01 μM. Besides, based on Liu

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et al., mentioned that chloroquine is stronger that hydroxychloroquine in waiting for the virus duplication based on the E6 Vero test in African green monkey's kidney and resulted in CC50 of chloroquine and hydroxychloroquine is 273.20 μM and 249.50 μM. But, both of them are known can inhibit the SARS-CoV-2 virus duplication (Pastick et al., 2020).

CONCLUSION

The Coronavirus is one of the diseases in which its infection spread is very fast which is through the respiratory tract so that it can cause a syndrome of symptoms acute breathing. The genome similarity between SARS-CoV-2 and SARS-CoV becomes strong proof that SARS-CoV-2 comes from the bats. By in vitro, chloroquine and hydroxychloroquine from 4-aminoquinolin is very effective in controlling the COVID-19 infection that has a function in inhibiting enzyme needed by the virus to duplicate themselves. The chloroquine treatment can be given to the patient who is diagnosed as pneumonia COVID-19 in the degree of mild, moderate, and severe without any contraindication towards chloroquine dose is 3x200 mg in 10 days but the treatment can be varied from 5 to 20 days based on the clinical degree especially in respiratory disorder.

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Research Article

Comparison of clinical evaluation of post-operation patients of open reduction internal fixation (ORIF) plating proximal humerus using conventional methods and minimally invasive plate osteosynthesis (MIPO) in Surabaya

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Abstract

There are several kinds of approaches in the installation of implants for proximal humerus therapy. At present, minimally invasive surgery is gaining in popularity; this is supported by increasingly good technological developments to optimal the postoperative outcome is more optimal than conventional methods. This study used an analytic retrospective design with samples of post-ORIF Plating MIPO and posted ORIF due to proximal humeral fracture. Evaluation using the instrument of VAS Score, ASES Score, and measurement of range of motion.

Statistical tests showed that there was a significant difference in the VAS score at the first evaluation ($p = 0.002$); the last review was not significant. In ASES Score, abduction, flexion, and external rotation, there were significant differences during the first and last evaluations. Adduction, extension, and internal rotation have no significance. The method of minimally invasive plate osteosynthesis (MIPO) on proximal humeral operative fracture therapy had a better clinical outcome and operating time than post-ORIF plating with conventional methods. As well, the MIPO method on proximal humeral fracture operative therapy based on radiological features has the same union rates compared to post ORIF plating with conventional methods. The method of minimally invasive plate osteosynthesis (MIPO) in operative therapy of neer 2 and 3 proximal humeral fracture has a better clinical outcome than conventional methods.

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INTRODUCTION

Proximal humeral fracture is one of the most common fractures in osteoporosis patients with an incidence rate of 63 to 105 fractures per 100,000 populations each year (Bucholz et al., 2010; Solomon, Nagayam, and Warwick, 2010). There are many choices of therapeutic modalities that can be used for proximal humeral fractures depending on the severity, in general, the management of the fracture can be divided into two, namely through nonoperative and operative, 80% proximal humeral fracture is non-displaced or minimally displaced fracture so that it can be treated nonoperatively (Maier et al., 2014).

There are several kinds of approaches in implant installation for proximal humerus therapy, both with conventional to minimally invasive operating techniques. At present minimally invasive surgery is a popular technique that is supported by the development of improved imaging radiographic technology and the emergence of mono / polyaxial locking plate, the hope, of course, is that the postoperative outcome which is more optimal than the conventional method is the decrease of soft tissue trauma which leads to a decrease in the ratio of complications, reduced postoperative pain, less periarticular adhesion, and better joint function (Ruchholtz et al., 2011; Ismail et al., 2012)

Therefore, in this study we aim to compare the functional outcome method with the conventional method that has been used so far, it is hoped that this study can provide additional references, especially in handling proximal humeral fractures.

METHODS

This study used a descriptive, retrospective study design—the sample of the study that met the criteria for sample acceptance and rejection consisting of 18 samples. The sample used was posted ORIF Plating MIPO and post ORIF patients. The conventional Plating Approach was caused by a proximal humeral fracture that matched Neer's criteria (Figure 1) in RSUD Dr. Soetomo from January 2015 to September 2018 and is willing to be the subject of research and fulfill the inclusion criteria.

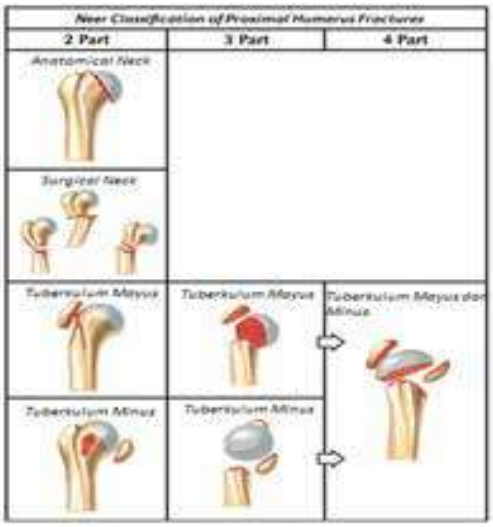

Figure 1. Neer's Classification in humerus proximal fracture (Thompson JC, 2010)

The inclusion criteria in this study posted ORIF Plating MIPO and posted ORIF patients. The conventional Plating Approach was caused by proximal humeral fractures that matched the criteria of Neer 2 and 3 with a surgical evaluation time of three months as many as two evaluation times with a range of evaluation is three months. Patients are willing to be the subjects of the study. Exclusion criteria in this study are patients with upper extremity neurological disorders and patients with multi fractures around the shoulder joint. The sampling technique was carried out by taking patient data post-ORIF Plating MIPO or post ORIF Plating conventional Approach caused by proximal humerus fractures according to Neer's criteria at Dr. General Hospital. Soetomo in the city of Surabaya. Selected patients will be compared clinical evaluations using a Visual

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Analog Scale (VAS), American Shoulder and Elbow Surgeon (ASES) Score questionnaire, and range of motion (ROM) measurements. The sample size was carried out by taking data post-ORIF Plating MIPO and post ORIF conventional Plating Approach, caused by proximal humeral fractures that matched Neer's criteria at RSUD Dr. Soetomo. All the data patients were evaluated. The nonparametric Wilcoxon and Mann-Whitney-U test were used to determine significant differences between samples. $P < 0.05$ was taken as an indication of significance. All statistical analyses were performed using SPSS version 22.0 software. The ethics committee approved this research of RSUD Dr. Soetomo Surabaya with ethics number 0967 / KEPK / II / 2019

RESULTS

In this study, there were 18 samples with a ratio between men and women of 1:1. The youngest patient is 30 years old, and the oldest is 78 years old, with an average sample of 50 years old. The detail of the sample characteristics was shown in Table 1.

The selected samples were operated with two treatments, namely MIPO and Conventional. In this study between conventional MIPO vs. normality test tests with various variables, among others, measuring pain (Table 2), ASES score and movement in the humeral extension, internal rotation, and external rotation, after that the abnormal distribution results in all of these variables except for the abduction variable obtained values with normal distribution, $P > 0.05$ so to test whether there is a difference between conventional vs. MIPO on variable abduction using Independent Sample t-Test while variables with abnormal distribution using Mann Whitney. The statistical results were shown in Table 3.

Table 1. Characteristics of the sample.

Characteristics	Number of Samples (percent)
Gender	
Male	9 (50%)
Female	9 (50%)
Age (Mean ± SD)	50.50 ± 13.61
30-45	8 (44,4%)
46-60	5 (27,8%)
>60	5 (27,8%)

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Tabel 2. VAS comparison between MIPO and conventional.

	Type of treatment	Mean±SD
VAS 1st evaluation	MIPO	2.33±0.71
	Conventional	3.89±0.78
VAS final evaluation	MIPO	0.44±0.53
	Conventional	0.44±0.53

Tabel 3. Hypotesis statistic test

Variable		Mean±SD	P value (95%CI)
VAS Score	Initial Evaluation	2.14±0.37	0.002
	final evaluation	0.42±0.53	0.916
ASES Score	Initial evaluation	92.62±1.62	0.000
	final evaluation	97.70±0.66	0.004
Abduction	Initial evaluation	92.85±9.51	0.007
	final evaluation	121.42±21.15	0.017
Adduction	Initial evaluation	48.57±3.77	0.740
	final evaluation	48.57±3.77	0.740
Extension	Initial evaluation	55.71±5.34	0.638
	final evaluation	58.57±3.77	0.913
Flexion	Initial evaluation	94.28±5.34	0.018
	final evaluation	125.71±20.70	0.010
Internal rotation	Initial evaluation	54.28±7.86	0.880
	final evaluation	60.00±3.77	0.309
External rotation	Initial evaluation	68.57±3.77	0.014
	final evaluation	68.57±3.77	0.014

DISCUSSION

In this study, an assessment of the VAS score was conducted between the use of MIPO and conventional. It was found that the average VAS score on the use of MIPO was 2.3 and conventional at 3.8 in the first evaluation and 0 in the use of MIPO and conventional at the last evaluation. In this study, there was a significant difference in the VAS score between the use of MIPO and conventional measures at the first evaluation (p = 0.002). Still, at the last evaluation, there were no differences in the VAS scores in the two groups (p = 0.916). This is similar to the research conducted by Liu et al., which states that there are differences in VAS scores between the use of MIPO and conventional (p = 0.02) (Liu et al., 2016). No complication happened to the patient with these two types of surgery.

ASES (American Shoulder Elbow Surgeon Score) in this study was also investigated to determine the differences between the use of MIPO and conventional actions. In this study, there were significant differences in the two actions if they were associated with the ASES score. In the first evaluation, the average ASES Score of MIPO users was 89.8, and the conventional was 82.4 (p = 0.014). Whereas in the last evaluation, the average ASES score of patients with MIPO users was 97, and conventional was 95 (p = 0.029).

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This is the same as research by Shang et al. (p = 0.001) using the ASES Score, which states that there are significant differences in ASES Score between MIPO and conventional. It used another functional outcome score method, Constant-Murley, which also showed significant functional outcome differences (p = 0.03) (Shang LP et al., 2013). This result is different from the research conducted by Esmailiejah and Yu B et al., where there were no significant differences in functional outcomes between patients operated on using MIPO with conventional methods (Esmailiejah AA, 2015; Yu et al., 2016)

In this study, it was conducted to determine ROM function both in the ROM function of the initial action and after the action, including ROM studied, abduction, adduction, flexion, extension, internal rotation, and external rotation. In this study, between conventional vs. MIPO actions, there was a significant relationship between the initial and final evaluation ROM in ROM, namely adduction and abduction. ROM on abduction in this study with a number of angles of 110/120 in which the initial ROM evaluation was 110 degrees and finally 120 degrees with the use of MIPO was in line with the study supported by Zhou ZB et al., with a mean ROM of MIPO measures of 110-180 degrees and there was a match in the Vochteloo et al.'s study, et al. mentioned after the conventional operative action obtained an abduction angle of 90 degrees, wherein this study conventional measures obtained an abduction angle of 90 degrees (Vochteloo and Krekel, 2011; Zhou, Gao, and Tang, 2012).

But in this study, after being tested statistically Mann Whitney U and Wilcoxon did not find a significant relationship to other ROM components both from flexion, extension, internal rotation, external rotation with two differences in working methods both with MIPO and with conventional handling both on evaluation ROM start and end.

One of the major difficulties of MIPO is obtaining adequate fracture reduction. During conventional ORIF, fracture reduction is achieved by direct visualization of the fracture and temporary stabilization with bone clamps. MIPO, on the other hand, requires indirect reduction techniques and closed fracture manipulation while plate fixation is obtained despite these challenges. Many factors can affect functional outcome between using conventional ORIF versus MIPO. Further research is needed.

CONCLUSION

Based on the discussion that has been described, the method of minimally invasive plate osteosynthesis (MIPO) on operative proximal humeral fracture therapy has a better functional outcome than post-ORIF plating with conventional methods using ASES score, because using MIPO less damage so that recovery and outcomes are faster and better.

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Research Article

Relationship of prostate-specific antigen (PSA) and prostate volume in patients with biopsy proven benign prostatic hyperplasia (BPH)

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ABSTRACT

Benign prostatic hyperplasia (BPH) is one of the most common benign tumors in men with prevalence ranging from 50% for men in their 60s to 90% for men in their 80s. The researcher sought to determine the relationship of prostate-specific antigen (PSA) and prostate volume in patients with benign prostatic hyperplasia. This study was based on 33 cases of benign prostatic hyperplasia in the Department of Urology, RSUD Dr. Soetomo Surabaya that diagnosed by histopathology examination. Cases with malignancy, acute urinary retention, and prostatitis were excluded. The variables of prostate-specific antigen and prostate volume were examined. The results of this study found that 33 men were enrolled with mean PSA 16,04 ng/ml and a mean prostate volume of 49,13 ml. Overall, 84,8% had PSA level >4 ng/ml and 90,9% had prostate volume >25 ml. Prostate-specific antigen has significant correlation with prostate volume (p=0,019; r=0,362). This study concluded that prostate-specific antigen and prostate volume showed a significant correlation.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a histological diagnosis associated with uncontrolled proliferation of connective tissue, smooth muscle, and epithelial glands of the prostate gland resulting in increased prostate volume (Parsons & Patel, 2014). More specifically, prostate enlargement occurs due to hyperplasia of the stromal and epithelial glands that occur in the periurethral transition zone of the prostate gland (Dhingra & Bhagwat, 2011). This condition causes the urethra pars prostatic depressed and it will inhibit the flow of urine when emptying the bladder. Disruption of urine flow in BPH causes symptoms such as frequency, urgency, nocturia, intermittence, decreased flow, and hesitation (Kapoor, 2012).

Genetic, dietary, and lifestyle factors play an important role in the growth process of BPH. Recent research shows that there is a strong association between clinical BPH and metabolic syndrome, erectile dysfunction, and inflammation (Lim, 2017). The incidence of BPH is very much related to age. Prostate enlargement begins when a man turns around 40 years old. The prevalence of BPH from histology shows positive results in approximately 8% of men in their 40s, 50% in their 60s, and 80% in their 90s (Lim, 2017).

Prostate volume is an indicator of BPH and progression therapeutic response given. Transrectal ultrasonography (TRUS) is the most accurate method for assessing the shape and volume of prostate examination. The TRUS examination includes a routine examination for BPH patients (Mochtar et al., 2015).

Serum prostate-specific antigen (PSA) tests are performed for health screening and early detection of prostate cancer. PSA measurement should be performed only if a diagnosis of prostate cancer will change the management or if PSA can assist in decision-making in patients at risk of progression of BPH (Mochtar et al., 2015). Serum PSA is a marker of prostatic gland epithelial cell activity. Increased serum PSA levels often indicate abnormalities of the prostate (Kowalkouski, Goltz, Hart, & Latini, 2013). This shows that in addition to prostate volume, serum PSA also has a relationship with BPH in terms of predicting progression. Much research has been done in various countries regarding the relationship between PSA and prostate volume, but not many similar studies have been done in Indonesia, especially in Surabaya. Our research aims to analyze the relationship between serum PSA and prostate volume in patients diagnosed with BPH through histopathological examination.

METHODS

This study was an observational analytic study with a cross-sectional approach conducted in the case of BPH at the Urology Polyclinic Dr. Soetomo Surabaya in January 2017 - December 2018. The inclusion criteria of this study were: (1) BPH was diagnosed based on histopathological examination results, (2) data on the results of PSA examination in medical records and (3) data on the results of the TRUS examination was recorded in medical records. Patients diagnosed with BPH with malignancy, acute urinary retention and / or prostatitis were excluded from the study data.

Serum PSA samples of patients were processed using ADVIA-Centaur XPT by the direct chemiluminometric method in the Clinical Pathology laboratory of Dr. RSUD Hospital Soetomo Surabaya. The prostate volume calculation formula used is a prolate ellipsoid. The formula used to calculate prostate size is height (anteroposterior) x width (transversal) x length (cephalocaudal) x (π / 6) (Haverkamp et al., 2019). Determination of the relationship between serum PSA and prostate volume using

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the Spearman statistical test with statistical analysis was significant when p <0.05. This study has received ethical approval from the Health Research Ethics Committee Dr. Soetomo Surabaya (0620 / KEPK / IX / 2018).

RESULTS

Samples were collected from 33 patients who met the inclusion and exclusion criteria. All patients were male with various age ranges. The average age, serum PSA and prostate volume are shown in Table 1.

The average age of patients diagnosed with BPH was 64.81 years (SD 7.06, age range 53-80 years), 2 (6.1%) were in the age range 46-55 years, 20 (60.6%) were in the age range of 56 - 65 years and 11 (33.3%) were in the age range> 65 years. The relationship between age, serum PSA and prostate volume of 33 BPH patients is summarized in Table 2.

The average serum PSA was 16.04 ng/dl (SD 6.23, serum PSA range 2.20 - 80.40 ml). The majority of serum PSA BPH patients (84.8%)> 4 ng/dl. The average prostate volume is 49.13 ml (SD 18.60, volume range 14.3 - 95.7 ml). The majority of BPH patients (90.9%) have prostate volume> 25 ml. There was a strong relationship between serum PSA and prostate volume (Spearman correlation coefficient r = 0.362, p <0.05). The relationship between serum PSA and prostate volume in BPH patients are shown in Table 3.

Table 1. The main parameters of the study

Parameter	Mean	SD
Age	64,81 years	7,06
PSA serum	16,04 ng/dl	16,23
Prostate volume	49,13 ml	18,60

Table 2. Average research data by age group

Age (years)	Number of patients [%]	PSA (ng/dl) [SD]	Prostate volume (ml) [SD]
46 – 55	2 [6,1]	12,50 [4,89]	53,50 [12,59]
56 – 65	20 [60,6]	18,29 [18,57]	53,12 [18,66]
>65	11 [33,3]	12,59 [12,63]	41,08 [18,00]
Total	33 [100]	16,04 [6,23]	49,13 [18,60]

Table 3. Spearman correlation test between PSA levels and prostate volume

PSA and prostate volume correlation	Result
Correlation coefficient	0,362
Significance (*one-tailed*)	0,019

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DISCUSSION

A total of 33 data from patient medical records have been verified as inclusion data from this study. This study found that the majority of BPH events occurred in the age range of 56 - 65 years, with an average age of 64.81 years. These results are consistent with research conducted by Al-Khalil et al. (2016), of the 130 BPH cases found, 86 were aged 66-69 years. Another recent study by Krisna, Maulana, & Kresnoadi (2017) stated that the average age of BPH patients in their study was 64.32 years. These findings indicate that the risk of BPH events in men increases in old age.

This study showed that as many as 84.8% of BPH patients had PSA levels exceeding the normal limit (> 4 ng/dl) and the remaining 15.2% had PSA levels within the normal range (≤4 ng/dl). These results are consistent with the results of research conducted by Liu, Tang, Gong, & Kong (2017) which states that BPH patients with PSA levels> 4 ng/dl are more numerous than BPH patients with PSA levels ≤4 ng/dl both before surgery and after surgery. Other studies that also support this result are studies by Rybalov et al. (2013) which states that 171 of the 245 BPH patients studied had PSA levels> 4 ng/dl. In another similar study conducted by Erdogan et al. (2019), the average PSA level was 9.3 ng/dl from a total of 137 BPH data obtained. Research conducted in Indonesia by Putra et al. (2016) on 1,638 BPH patients found an average PSA level of 4.93 ng/dl. This study obtained data with an average PSA level that is far different from the existing research, with a difference of about 11.1 - 6.73 ng/dl. This difference is due to the different research methods used. In a study conducted by Erdogan et al. (2019) has exclusion criteria for PSA levels of more than 30 ng/dl. In a study conducted by Putra et al. (2016) established exclusion criteria for PSA levels of more than 10 ng/dl. Research conducted at RSUD Dr. Soetomo uses a different method from the research mentioned above. The difference is that in this study, there is no maximum limit of PSA levels in the exclusion criteria so that the research results obtained are not the same as the existing research.

This study showed that as many as 90.9% of BPH patients had a prostate volume exceeding the normal limit (> 25 ml), and the remaining 9.1% had a prostate volume within the normal limit (≤25 ml). These results are consistent with research conducted by Deori, Das, & Rahman (2017) which states that 27.5% of BPH patients have prostate volume ≤30 ml, while the remaining 72.5% have prostate volume> 30 ml. The highest volume frequency distribution is in the range of 40 - 60 ml, as many as 13 patients (39.4%). The average prostate volume in this study was 49.68 ml. Research conducted by Mao et al. (2009) in China stated that the average prostate volume of 268 BPH patients was 42.45 ml. Putra et al. (2016) research in Indonesia states that the average prostate volume of 666 BPH patients with indwelling catheters is 47.58 ml, while the average prostate volume of 972 BPH patients without indwelling catheters is 41.43 ml. The difference in the average prostate volume in BPH patients can be due to BPH risk factors that are not the same in each study. These risk factors include race (Egan, 2016), obesity (Jung et al., 2016) and diabetes (Sarma et al., 2009). The above risk factors are not mentioned Mao et al. (2009) and Putra et al. (2016) can be the cause of the difference in average prostate volume results when compared to the results of research conducted at Dr. Soetomo Hospital.

Research conducted at Dr. Soetomo Hospital Surabaya found a significant correlation between PSA levels and prostate volume in BPH patients ($r = 0.362$; $p = 0.019$). Another study supporting this result is Putra et al. (2016), who found a correlation between PSA levels and prostate volume in the male population at Dr. RSUPN Cipto Mangunkusumo hospital ($r = 0.26$; p

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= 0.0001) of the 1,638 BPH patients studied. Research Krisna, Maulana, & Kresnoadi (2017) states that there is a correlation between PSA levels with the prostate volume of 0.384 (p <0.05) in BPH patients studied at Bhayangkara Hospital Mataram. Another study by Mao et al. (2009) suggested that there was a correlation between PSA levels and prostate volume (r = 0.278; p <0.001) in 268 Chinese men with a diagnosis of BPH.

A significant relationship between PSA levels and prostate volume, can be supported by one of the pathophysiological theories of BPH, namely the presence of epithelial-stromal interaction. Epithelial-stromal interaction plays a vital role in the hormonal, cellular, and molecular regulation of prostate development in both normal and neoplastic prostates (Tang & Yang, 2009). The aging process causes a gradual accumulation of prostate mass as a result of ongoing prostate gland-stromal interactions, which can be increased by various growth factors that are provided systemically through circulation or locally through the urethra (Tang & Yang, 2009). Expressions that deviate from growth factor peptides or their receptors can directly contribute to uncontrolled growth which ultimately results in BPH. Stromal cells are responsible for secreting many growth factors such as fibroblast growth factors, growth factors such as insulin I and II, and tumor growth factors, which act automatically on the stroma itself and adjacent glandular cells to induce proliferation (Tang & Yang, 2009). The proliferation of these cells causes excessive prostate volume increase in BPH patients, causing clinical manifestations that interfere with and reduce the quality of life of patients.

There are several limitations to this study. First, we evaluated PSA and prostate volume-based only on medical record data. Therefore, our sample size depends on the completeness of the data written in the medical record. Second, prostate parameter data is only taken at one time. The best way to monitor the prostate growth pattern would be to perform a longitudinal study, where all of the participants could be measured and followed for several years in order to obtain an accurate growth rate (Zhang et al., 2013). Longitudinal research in the future is expected to complement the results of this cross-sectional study.

CONCLUSION

In conclusion, serum PSA levels have a strong relationship with prostate volume in BPH patients. The average serum PSA level and prostate volume obtained in this study are slightly different from studies that have been done in several other areas before. The researcher suggests that further research be conducted on BPH in Indonesia.

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Research Article

Sequestration of erythrocytes infected with *Plasmodium berghei* ANKA in BALB/c mice treated with goat bile

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Abstract

Sequestration of *Plasmodium berghei* ANKA-infected erythrocytes occurs in BALB/c mice as characteristic of *Plasmodium falciparum* infection in humans. Animals' bile has been widely used for centuries in Traditional Chinese Medicine. Goat bile has been used in healing infectious and non-infectious diseases; however, no report on the use of goat bile against malaria infection and sequestration. The purpose of this study was to analyze the correlation between parasitemia and sequestration in the liver of *P.berghei* ANKA-infected BALB/c mice treated with goat bile. This research was an in vivo experimental study using the post-test control group design. The male BALB/c mice aged ± 6 weeks, body weight 20-25 g were used. The mice were divided into five groups where Group 1-3 were mice treated with goat bile 25%, 50%, and 100%, respectively. Group 4-5 were negative (sterile water) and positive controls (DHP). Parasitemia was observed daily from each mouse and the number of sequestered infected erythrocytes on the endothelium of sinusoids. The data were analyzed using t independent test. Antimalarial activity of goat bile was shown by the lower parasitemia in goat bile-treated mice compared with the negative control. The average number of sequestration was goat bile concentration-dependent manner. The higher the concentration, the lower the number of sequestration. Sequestration was correlated with parasitemia (p=0,0001). Sequestration of *P.berghei* ANKA-infected erythrocytes correlated with parasitemia, and was goat bile concentration-dependent manner.

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INTRODUCTION

Malaria is a disease that remains a problem in the world, especially in endemic areas, such as Africa, Southeast Asia, and the Eastern Mediterranean. According to the World Malaria Report 2018, during 2017, there were 219 million new cases of malaria with a mortality rate of 435,000 worldwide (WHO, 2018). In Indonesia, the hyperendemic malaria areas were provinces of Papua, Maluku, North Maluku, and East Nusa Tenggara (Pusdatin, 2016).

Protozoan genus *Plasmodium* causes malaria. In general, five species of *Plasmodium* that infect humans are *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi* (Wassmer et al., 2015). Four *Plasmodium* species infecting rodents that have been extensively used in *in vivo* rodent research are *Plasmodium berghei, Plasmodium chabaudi, Plasmodium yoelii, and Plasmodium vinckei* (De Niz & Heussler, 2018).

Clinical pathologies of *P. falciparum* in human infection are severe anemia (White, 2018), sequestration of infected erythrocytes (David et al., 1983), rosetting, and organ complications such as cerebral malaria, malaria in pregnancy, splenomegaly, hepatomegaly, hypoglycemia, pulmonary edema to death (Bartoloni & Zammarchi, 2012). Hepatomegaly is a common feature in malaria infection, especially in *P. falciparum* infection (Viriyavejakul et al., 2014). Sinusoidal dilatation is the most important factor contributing to the enlargement of the liver (Baheti, Laddha, & Gehlot, 2003). Sequestration is a characteristic of *P. falciparum* infection where infected erythrocyte as adhere to endothelial cells in microvasculature of vital organs such as the brain, lungs, spleen, placenta, eye, subcutaneous fat, heart, bone marrow, intestine, liver which can cause various types of malaria severity (Brugat et al., 2014). Sequestration of *P. falciparum* in small blood vessels induces local blood flow impairment leading to disturbances and failure in various organs, including liver (MacKintosh, Beeson, & Marsh, 2004).

Similar clinical features are found in rodent malaria. Experimental cerebral malaria (ECM) in C57BL/6 mice infected with *P. berghei* ANKA showed sequestration of infected erythrocytes in the brain, as found in human cerebral malaria (Baptista et al., 2010). Sequestration of *P. berghei* ANKA-Infected erythrocytes in BALB/c mice are found in the spleen, lungs, and adipose tissue indicated that sequestration is associated with the severity of the disease (Franke-Fayard et al., 2010). Sequestration usually occurs when erythrocytes infected with the stages of adult trophozoites, schizonts, and young gametocytes (Mota & Rodriguez, 2017) as an attempt to escape from the immune system (Belachew, 2018). In fact, sequestration in *P. berghei*-infected mice evidenced by the presence of schizont-infected erythrocytes sequestration in the organ that expressing CD36+ markers (Franke-Fayard et al., 2010).

Animals' bile has been widely used for centuries in Traditional Chinese Medicine (TCM) for clinical practice (Li et al., 2016). Bile is secreted from hepatocyte involves in biliary system (Hundt M et al, 2018). Bile contains about 95% of water, bile salts, phospholipid bilirubin, cholesterol, amino acids, steroids, enzymes, porphyrins, vitamins, and heavy metals, and exogenous drugs, xenobiotics and toxic environments (Boyer, 2013) and a wide variety of antioxidants, bilirubin, glutathione, vitamin E, and melatonin (*N*-acetyl-5-methoxytryptamin) (Wang & Carey, 2014). The functions of bile are to improve liver function, dissolve gallstones, inhibit bacterial and viral multiplication, promote cardiac chronotropsim,

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as well as exhibiting anti-inflammatory, anti-pyretic, anti-oxidant, sedative, anti-seizure, anti-allergic, anti-congestive, anti-diabetic and anti-plasmodic effects (Boyer, 2013).

Malaria control in Indonesia uses Artemisinin-based combination therapy (ACT) as recommended by WHO (Kemenkes, 2016). However, some people of Indonesia consume goat gallbladder to treat malaria and to increase their stamina (Amalia, 2012). Goat bile has been used in healing several diseases such as optical atrophy, blindness, and diarrhea (Li et al., 2016; Wang & Carey, 2014). Until now, there is no report on the effect of goat bile to sequestration of malaria parasite-infected erythrocytes in the vital organs of infected mice. The effect of goat bile on the sequestration of *P.berghei* ANKA-infected erythrocytes in the liver of BALB/c mice is reported herein.

METHODS

Ethical approval

The proposal of this research has been reviewed by the Ethics Committee of Faculty of Medicine, Universitas Airlangga as described on the Ethical Clearance No. 110/EC/KEPK/FKUA/2019.

Research Design

This research is an in vivo experimental study using the post-test control group design. After infected with *P. berghei* ANKA mice were divided into five groups. Group 1 was a positive control treated with 187.2 mg/kg body weight of Dihydroartemisinine Piperaquine or DHP (Mersi Farma, Sukabumi, Indonesia), Group 2 was negative control mice were only given with sterile water, Group 3-5 were given with 25% (GB25), 50% (GB50) and 100% goat bile (GB100), respectively.

Parasite infection in mice

Parasite used in this experiment was *P. berghei* ANKA obtained from the Department of Medical Parasitology, Faculty of Medicine, Universitas Airlangga. The BALB/c mice aged six weeks with average weight about 25 grams, healthy, and had never received any treatment before. Mice were acclimatized for one week before infection. Five donor mice were infected with 200µL per mouse of *P. berghei* ANKA-infected frozen blood. When parasitemia reached ±20% mice were sacrificed, the blood was collected by cardiac puncture and infected to test mice. Each test mouse was infected with $1x10^6$ infected erythrocytes. A four day-treatment was started on day two post-infection. Each mouse was given 0,5 mL/25-gram mouse of each concentration of goat bile.

Goat bile and DHP preparation

Goat gallbladders were bought from Pegirikan slaughterhouse Surabaya. The healthy Java goat was chosen as this strain of goat was usually consumed by the Javanese. Gallbladders were sprayed with 70% alcohol before bile removal with syringe. Goat bile were then pooled into sterile tube and diluted with sterile water to prepare 25% and 50% goat bile solutions. The working goat bile solutions were stored in a refrigerator during the course of experiment. The DHP was diluted with sterile water to prepare 187.2 mg/kg body weight of doses.

Determination of parasitemia

Parasitemia of infected donor and test mice were determined daily by counting the infected erythrocytes on Giemsa-stained thin blood smears of mouse tail blood. Parasitemia was calculated using the following

$$\text{formula} = \frac{\text{number of infected erythrocytes}}{\text{total number of erythrocytes}} \times 100\%$$

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Observation of sequestration

Test mice were anesthetized by intraperitoneal injection of ketamine prior to liver removal. Livers were then fixed in 10% formaldehyde. Fixed organs were embedded in wax, sectioned (5µm), and stained with hematoxylin eosin HE. The sequestrations of P. berghei-infected erythrocytes on endothelial cells of liver microvasculature were observed quantitatively on 10 fields of view or 100 sinusoids microscopically at 1000x magnification (Olympus CX21, Tokyo, Japan).

Statistical analysis

The difference of the parasitemia and the number of sequestrations were compared with negative and positive controls were analyzed using t dependent test. The correlation of parasitemia and number of sequestration was analyzed using Pearson correlation test.

RESULTS

Parasitemia

Based on **Figure 1,** normal parasitemia was shown in negative control which did not received any drug administration. On the other hand, parasites in mice treated with DHP were completely eliminated, indicated a potent anti-malaria drug. There was no significant difference of parasitemia between the negative control and GB treatment group (p>0,05). However, parasitemia of the GB25, GB50 and GB100 were lower than that of negative control. This result indicated that GB possessed antimalarial activity.

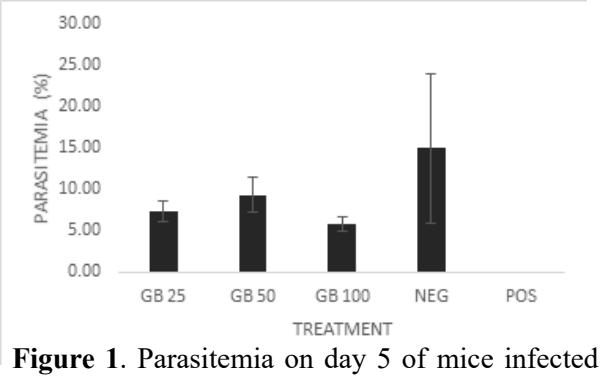

Figure 1. Parasitemia on day 5 of mice infected with *P. berghei* ANKA treated with goat bile compared with positive and negative controls. GB25: goat bile 25%, GB50: goat bile 50%, GB100: goat bile 100%. NEG: negative control (sterile water). POS: positive control (187.2 mg/kg body weight of DHP).

Sequestration

Figure 2 shows the average number of sequestrations of *P. berghei* ANKA-infected erythrocytes decreased along with the increase of concentration of goat bile. Statistical analysis of sequestration was shown in **Table 1**. The difference of sequestration in the liver of mice treated with GB25 and GB50 was not significant compared with negative controls (p>0.05), while GB100 was significant. In contrast, the comparison between positive controls with GB25, GB50 showed significant differences (p <0.05). However, there was no significant difference between positive control and GB100. The significant difference was obviously seen between negative and positive controls. The sequestration of *P.berghei* ANKA-infected erythrocytes in current research showed that BALB/c mice

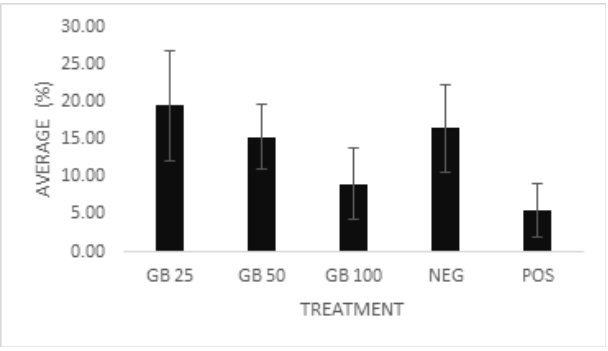

Figure 2. Sequestration of *P. berghei* ANKA-infected erythrocytes treated with goat bile compared with positive and negative controls. GB25: goat bile 25%, GB50: goat bile 50%, GB100: goat bile 100%. NEG: negative control (sterile water). POS: positive control 18.72 mg/kg body weight.

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without goat bile treatment (negative control) similar to that of mice treated with GB25 (p=0,450) and GB50 (p=0,702, **Table 1**) where average number of sequestrated *P.berghei* ANKA-infected erythrocytes in negative control, GB25, and GB50-treated mice were 16.5,19.05 and 15.3, respectively. Sequestration in mice treated with GB100 was significantly different with the negative control.

The sequestration in the liver of mice is shown in **Figure 3**. This figure proved the sequestration of *P. berghei* ANKA-infected erythrocytes that occurred in the liver of BALB/c mice. Kupffer cells, hemozoin particles, and clumps also adhered to the liver endothelium. The Pearson correlation test for the correlation between sequestration and parasitemia resulted in a significant correlation with p=0.001 (significance at p<0.01).

Table 1. The average number of sequestration of *P.berghei* ANKA- infected erythrocyte in BALB/c mice liver treated with goat bile compared with negative (NEG) and positive (POS) control group

Group of mice		Mean± SD	p
NEG	GB 25	19.5 ± 7.314	0.450
	GB 50	15.3 ± 4.320	0.702
	GB 100	9 ± 4.733	0.034*
	POS	5.5 ± 3.620	0.003*
POS	GB 25	19.5 ± 7.314	0.002*
	GB 50	15.3 ± 4.320	0.002*
	GB 100	9 ± 4.733	0.181
	NEG	16.5 ± 5.822	0.003*

Statistical analysis using independent sample t test, n = 5.
*Significance p< 0.05

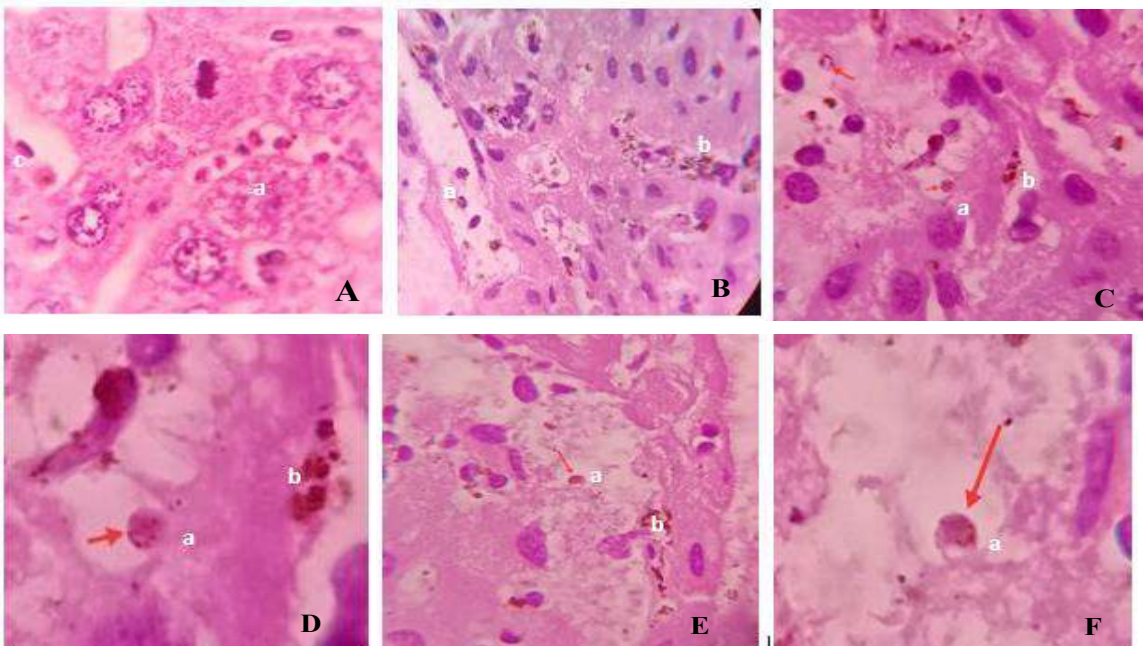

Figure 3. Representative photomicrograph of HE-stained *P. berghei* ANKA-infected erythrocytes sequestration in the liver of mice: A and B: several infected erythrocytes in sinusoid; C and E: infected erythrocytes sequestered on endothelium; D zoomed of picture C; F zoomed of picture E. a. infected erythrocytes adhered to endothelial (sequestration), b. Hemozoin, c. Kupffer cell.

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DISCUSSION

The effects of goat bile on the alteration of parasitemia and the average number of infected erythrocyte sequestration have been observed in this study. **Figure 1** shows the effect of goat bile on parasitemia in mice treated with GB25,GB50, and GB100, which were lower than that in negative control. This result indicated that goat bile possessed antimalarial activity against *P.berghei* ANKA in BALB/c mice. Parasitemia in positive control was the lowest among GB-treated mice that reached to zero, indicating that DHP is a potent antimalarial drug. Bile acids are potent stimulators of suicidal erythrocyte death (eryptosis) in vitro because bile acid can induce the stimulation of Ca^{2+} entry (Lang et al., 2016). The low parasitemia may caused by eryptosis due to the entry of Ca^{2+}. Then, the erythrocytes lysed and lead to parasite malnutrition.

The higher average number of sequestration of *P.berghei* ANKA-infected erythrocyte in 100 sinusoids was shown in the GB25 treated mice that werenot significantly different compared with negative control, indicated the slight effect of goat bile to the sequestration. However, GB100 gives the effect significantly different similar to DHP in positive control that reduced sequestration of *P.berghei* ANKA-infected erythrocytes in the liver of BALB/c mice. These results suggested that the effect of goat bile to sequestration *P.berghei* ANKA-infected erythrocytes was a concentration-dependent manner.

Sequestration is a unique phenomenon that usually occurs in *P.falciparum*-infected erythrocyte in humans. Some studies have reported that sequestration has also occurred in *P.berghei*-infected C57BL/6 mice, BALB/c mice, Wistar rats, and SHR/NCrIBR rats (Franke-Fayard, 2005). The *P.berghei* ANKA-infected erythrocyte adheres to the endhotelial cells of microvasculature through the CD36+ (Franke-Fayard et al., 2010) and ICAM-1 (Cunningham et al., 2017) receptors in C57BL/6 and BALB/c mice. The ligand on *P.berghei* ANKA-infected erythrocytes was unknown (Cunningham et al., 2017; Franke-Fayard et al., 2010). Bile acids have the ability to increase nitric oxide (NO) (Nakajima et al., 2000) lead to the reduction of adhesion molecule on endothelial cells (Gao et al., 2018). The higher concentration of goat bile increased NO and reduced the expression of the adhesion molecule caused the lower number of infected erythrocyte sequestration.

Bile acids play a dual role due to their amphiphatic properties, which are hydrophobic and hydrophilic. Hydrophobic bile acids are strong cytotoxic acids, fully ionized at physiological pH values (Begley et al., 2005). The greater hydrophobicity the greater cytotoxic effect (Hofmann & Eckmann, 2006). The cytotoxic effect is played by hydrophobic deoxycholic acid (DCA) and chenodeoycholic (CDCA). Hydrophilic bile acids are cytotoxic inhibitor, which played by ursodeoxycholic acid (UDCA) and tauroursideoxycholic acid (TUDCA) (Hofmann & Eckmann, 2006; Mello-Vieira et al., 2013). The DCA, CDCA, and TUDCA increased Ca^{2+} in a concentration dependent manner (Nakajima et al., 2000).

CONCLUSION

Goat bile antimalarial activity in BALB/c mice infected with *P. berghei* ANKA and sequestration of infected erythrocytes in a concentration-dependent manner suggested that goat bile is a potential antimalarial therapy that may developed into a potent antimalarial drug through a series of more specific and intensive research.

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Research Article

Antimalarial activity of goat bile against *Plasmodium berghei* ANKA infection in BALB/c mice

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Abstract

Goat bile has been used by some Indonesian people to treat malaria and increase their stamina. This study aimed to prove whether goat bile toxic or not in BALB/c mice and to verify the antimalarial activity of goat bile at various concentrations in mice infected with *Plasmodium berghei* ANKA. Acute toxicity test was performed using twenty male BALB/c mice with an average body weight of 25 grams, which were divided into four groups. Mice were given 25%, 50%, and 100% goat bile, respectively, while negative control was given distilled water. Any change in weight, odor, color, agitation, appearance, color of urine and feces, coma, and death, were recorded. A different set of mice were infected with *P. berghei* ANKA. This study conducted using the posttest only control group design with four treatments and five replications. A four day-treatment of goat bile was given by oral gavage to find out its effect on parasitemia level. Infected mice were divided randomly into 4 groups, where the GBNeg group as negative control was given only distilled water. The GB25, GB50, and GB100 groups were treated with 25%, 50%, and 100% goat bile, respectively. The parasitemia was observed daily on Giemsa-stained tail blood smears of each mice. No death or other sign of toxicity was found in goat bile-treated mice. Goat bile showed anti-malarial activity. The parasitemia in all goat bile treated groups was lower compared with the negative control group. The ED50 of goat bile against the growth of parasite was 48,55 %. Goat bile is a potential source of new antimalarial therapies. Further investigations are recommended to yield new anti-malarial drug candidates.

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INTRODUCTION

Malaria is one of the deadliest infectious diseases worldwide. World Malaria Report 2018 estimated 219 million cases of malaria occurring worldwide and 435,000 death globally in 2017 (WHO, 2018). Indonesia is one of the countries with the highest malaria cases in Southeast Asia. Indonesia has 10.7 million inhabitants living in middle and highly endemic areas of malaria, especially Papua, West Papua, and NTT (Pusdatin, 2016). Novel approaches and new alternative malarial drugs are important to combat the disease (Zeleke et al., 2017) . The development of a new anti-malarial drug remains slow, with very little chemical diversity (Muluye et al., 2019).

Artemisinin-based combination therapy (ACT) such as dihydroartemisinin-piperaquine was used to treat malaria in Indonesia as recommended by WHO (Kemenkes, 2016). However, disease treatment in developing countries is often using ethno medicines, which regarded as primary choice as they are most affordable and accessible from available natural sources (Kitua & Malebo, 2004). The antimalarial drug effect is characterized by the inhibition of parasite growth (Penna-Coutinho et al., 2011). Ideal antimalarial candidates should also be able to prevent several conditions *in vivo* related to the parasite infection, including anemia and body weight loss (Zeleke et al., 2017a). Bodyweight is an indicator of metabolism and gut function. The decrease in body weight in mice infected with malaria may be a consequence of metabolic function disturbance, hypoglycemia or appetite depressant action of the mice (Basir et al., 2012; Fidock et al., 2004).

Traditional Chinese Medicine (TCM) has used various parts of the animal's body, including goat bile (Wang & Carey, 2014). Some people in Indonesia consumed goat bile to treat malaria and increase stamina. Goat bile has been found to have antimalarial activity against *P. berghei* ANKA in vivo and *P. falciparum* in vitro (Hapsari et al., 2014). So far, there is a lack of reports on the the antimalarial activity of goat bile in mice infected with *P. berghei* ANKA. The aim of this research was to find out antimalarial activity of goat bile in BALB/c mice infected with *P. berghei* ANKA.

METHODS

Ethical approval

The proposal of this research has been reviewed by the Ethics Committee of Faculty of Medicine, Universitas Airlangga as described on the Ethical Clearance No. 116/EC/KEPK/FKUA/2019

Preparation of goat bile

Goat gallbladders were obtained from the Pegirikan slaughterhouse in Surabaya, East Java. Goat bladders were sprayed with 70% alcohol before removing the bile by syringe and pooled into a sterile tube. Goat bile was then diluted with sterile water to prepare 50% and 25% solutions. The working goat bile was stored in the refrigerator during the course of treatment.

Acute toxicity test

Acute toxicity test of goat bile was performed using 25%, 50%, and 100% goat bile. Each mouse in each group was given 0.5mL/25 grams body weight using gavage for four days. (OECD, 2011). The mice in the control group were given 0.5 mL of distilled water. The mice throughout the period of the experiment were under careful watch. Any change in weight, odor, color, agitation, appearance and color of urine and feces, coma, and death were recorded. The body weight of each mice was measured and taken before treatment (D_0), at day 4 (D_4) and day 30 (D_{30}).

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Experimental design

Posttest only control group design was applied with a four day-treatment; each treatment consists of five replications. Mice were infected with $1x10^6$ of *P. berghei* ANKA-infected erythrocytes intraperitoneally. Mice were then randomly divided into four groups, where the GBNeg as the negative control group were given only distilled water. Group GB25, GB50 and GB100 were given with 25%, 50% and 100% goat bile, respectively.

Parasite and Infection Parasite used in this experiment was *P. berghei* strain ANKA obtained from the Department of Medical Parasitology Faculty of Medicine, Universitas Airlangga. Donor mice were infected with frozen *P.berghei* ANKA-infected mice blood. When parasitemia level reached 20%, the mice were sacrificed after ketamine anesthetized, blood was collected by cardiac puncture and infected to test mice. Each mouse was infected with $1x10^6$ of *P.berghei* ANKA-infected erythrocytes intraperitoneally.

Goat bile treatment

Goat bile treatment was given daily for four days starting on two days post-infection. Each mouse was given 0.5 mL/25-gram mouse orally of each concentration of goat bile. The mice in the negative control group were given distilled water with the same volume of goat bile.

Determination of Parasitemia

Thin smears were prepared on slides from the tail of infected mice. The slides were fixed with methanol and stained with 10% Giemsa, observed under light microscopy with 1000x magnification. The percentage of parasitemia was calculated using the following formula:

$$\% \text{ parasitemia} = \frac{\text{infected RBC}}{\text{total RBC}} \text{ x } 100$$

The percentage of the growth of parasite was calculated for each group using this formula:

$$\% \text{ growth} = \frac{P(d1-d0)+(d2-d1)+(d3-d2)+(d4-d3)}{(n-1)}$$

P(d): % parasitemia at day (d)
n: total treatment days.

The % inhibition of the growth of parasite was calculated for each group using the following formula.

$$\% \text{ inhibition} = 100\% - \frac{Pt}{Pc} \text{ x } 100\%$$

Pt: Parasitemia in treatment group
Pc: Parasitemia in control group

Data analysis

All treatments and control groups were considered for determination of fifty percent effective dose (ED50) of goat bile against *P. berghei* ANKA in BALB/c mice. The ED50 was determined by Probit analysis on SPSS 16.0 for Windows. P-values <0.05 were considered significant. The two tailed paired t-test was used to compared mean body weight before and after treatment. The level was considered significant at 95% confidence level and $p<0.05$.

RESULTS

Acute Toxicity

The physical sign of illness observed during the course of acute toxicity test was mild diarrhea, which occurs only within two days after initial treatment, and then the symptom was disappeared afterward. No death or other sign of toxicity was found in goat bile-treated mice. Figure 1 showed the control and treatment group progressively gain weight until the end of the experiment on day 30. A significant difference ($p < 0.05$) was observed

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GB50 treatment group in comparison with the negative control. There was no significant difference ($p < 0.05$) on day 4 of all treated groups compared with the negative group. A significant difference ($p < 0.05$) was observed on day 30 of treatment in the GB25 treatment group in comparison with the negative control.

Parasitemia

Figure 2 showed the percentage of parasitemia of mice infected with *P. berghei* ANKA. The parasitemia in all treated group were lower compared with negative control group. Significant difference ($p < 0.05$) was observed on day 1 of treatment in the GB100 group in comparison with negative control. There was no significant difference ($p < 0.05$) on day 2, 3, and 4 of all treated groups compared with the negative control. Overall, Figure 1 presents the percentage of parasitemia in *P.berghei* ANKA infected mice with treated group exhibiting lower percentage parasitemia level but a significant difference ($p < 0,05$) only occur on day 1 of treatment in the GB100 treatment group.

Table 1 showed the percentage of growth and inhibition of parasite in infected mice. The lowest percentage of parasite growth of 1,65% and a maximum percentage of parasite inhibition of 61,11% was shown in 100% goat bile treatment. Statistical analysis showed that the result of ED_{50} is 48,55%. This result showed that 48,55% of goat bile could inhibit 50% of parasite growth.

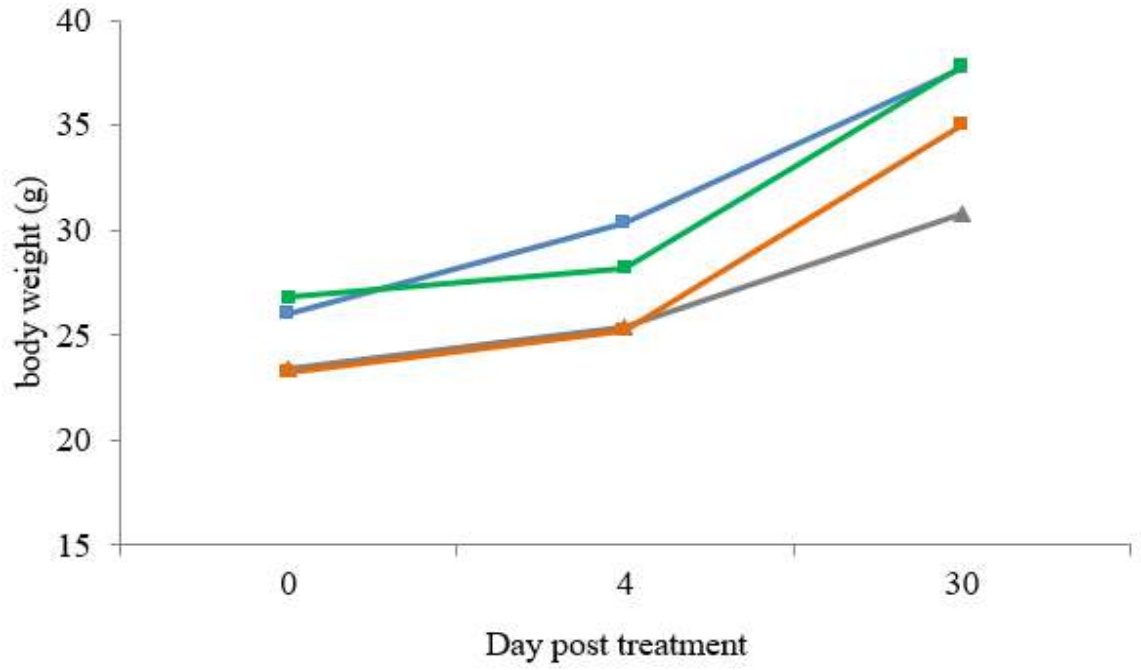

Figure 1. The Bodyweight of BALB/c mice treated with goat bile. GB25: goat bile 25%. GB50: goat bile 50%. GB100: goat bile 100%. NEG: Negative control.

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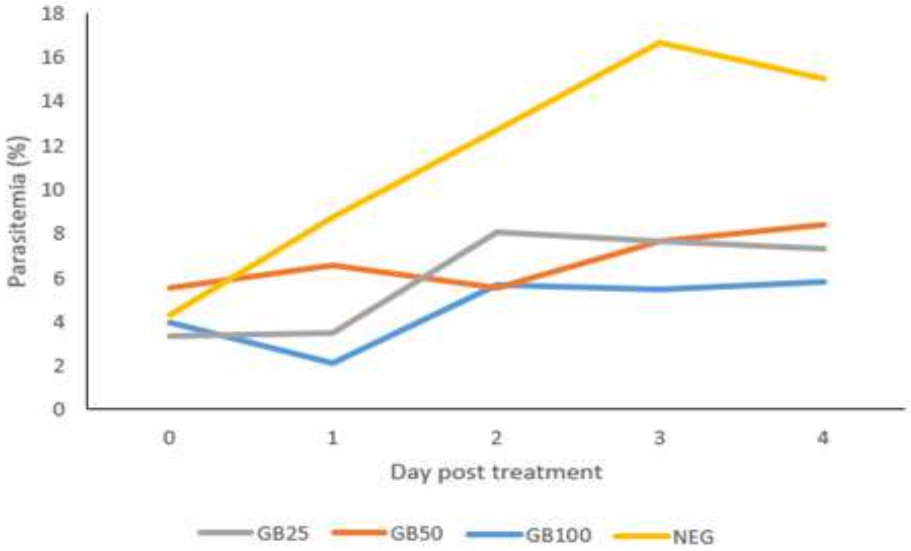

Figure 2. Percentage of parasitemia in *P.berghei* ANKA infected mice. GB25: goat bile 25%. GB50: goat bile 50%. GB100: goat bile 100%. NEG: Negative control.

Table 1. Percentage of growth and inhibition of parasitemia in *P.berghei* ANKA infected mice

Group	% Growth	% Inhibition
GB25 (goat bile 25%)	4,18 %	51,33%
GB50 (goat bile 50%)	2,42%	43,83%
GB100 (goat bile 100%)	1,65%	61,11%
GBNeg (distilled water)	11,78%	

DISCUSSION

The acute toxicity test resulted in mild intestinal toxicity, as shown by mild diarrhea in two mice treated with 100% goat bile. However, the increased of the mice body weights were not affected by goat bile treatment, indicated no significant toxicity of goat bile to intestinal effect. Body weight was measured to monitor the effects of treatment on factors such as metabolism and intestinal function (Basir et al., 2012). An intestinal disturbance may occur due to goat bile treatment that may affect intestinal metabolism. Metabolic function disturbance such as hypoglycemia and appetite depressant action of the mice (Basir et al., 2012; Fidock et al., 2004).

The *in vivo* evaluation proved the antimalarial activity of goat bile against *P. berghei* ANKA infection in mice. An ideal antimalarial candidate should prevent several conditions such as anemia, hemolysis, body temperature reduction, and body weight loss (Zeleke, Kebebe, Mulisa, & Gashe, 2017). The effect of the antimalarial drugs is characterized by the inhibition of parasite growth in the treatment group compared with a drug-free control group (Penna-Coutinho et al., 2011). The effects of goat bile on malaria infection in mice is shown by the percentages of inhibition and parasite growth in Table 1. The data shows the antimalaria activity of goat bile against *P. berghei* infection in BALB/c mice. Goat bile

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that the higher concentration affected the level of parasitemia compared with the negative control shown in Figure 2. Parasitemia level is influenced by various factors, including the host immune system (Kotepui et al., 2015). The density of parasites is one of the factors that influence the severity of clinical manifestations (Avrina et al., 2011).

Goat bile was one of the various animals' bile used in TCM. Even there was no report on the use of goat bile to treat infectious disease; goat bile has been believe to treat eye diseases, various infectious skin diseases, and constipation (Wang & Carey, 2014). Animal bile contains various compounds, including amino acids, steroids, enzymes, cholesterol, bile salts, bilirubin, phospholipids, vitamins, heavy metal, and environmental toxins. Goat bile contains taurocholate (TC), taurochenodeoxycholate (TCDC), taurodeoxycholate (TDC), glycocholate (GC), and monoglucuronide bilirubin pigment. About 5% of bile salts consist of organic and inorganic solutes. Dry matter of goat bile contains 15,7% protein (Boyer, 2013; Wang & Carey, 2014);

Although the active compound in goat bile for the antimalarial effect is not known or yet to be identified, bile acid and bile salt have known to have roles in therapeutic actions. Bear bile was used in TCM to reduce inflammation, liver diseases, and fever. Bear bile has taurine conjugate form of bile acid, such as taurochenodeoxycholic acids (TCDCA), taurodeoxycholic acids (TDCA), tauroursodeoxycholic acids (TUDCA), and taurocholic acids (TCA) (Coleman et al., 2006; Li et al., 2016). A role of bile acid in neuroprotection of age-related neurodegenerative disorder has been found (Ackerman & Gerhard, 2016). The beneficial effect of bile salt is as a natural detergent to prevent sexually transmitted disease and protection against bacterial growth (de Buy Wenniger & Beuers, 2010; Herold et al., 1999).

CONCLUSION

Although goat bile possessed mild intestinal toxicity in BALB/c mice, however, this result suggested that goat bile is one of the potential sources for new anti-malarial therapies. Further investigations are recommended, as there are no published studies on anti-malarial activity for specific compounds of goat bile that can yield new anti-malarial drug candidates.

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Research Article

Thrombocyte count in male and female adult of Dengue hemorrhagic fever patients

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Abstract

The trend of dengue virus infection in Indonesia has changed from children to older groups. Some studies suggested that different gender might affect the different progression of dengue infection, but its mechanism was unclear. This study analyzed the difference by evaluating the daily thrombocyte count pattern. An analytic observational study with a retrospective design was conducted using the secondary data collected from medical records in Dr. Soetomo General Hospital Surabaya during 2017-2018. The samples were adult patients in the age range of 18-55 years old with DHF but without comorbidity or coinfection. The total number of samples was 40 patients. The average of thrombocyte count in both male and female patients decreased since the 3rd day of illness and reached the lowest level on the 6th day of illness then increased on the 7th day but did not reach the normal range. Although the majority of thrombocyte count in females was lower than males, there was no significant difference in thrombocyte count pattern between them.

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INTRODUCTION

Dengue virus is classified as a Flaviviridae family, which has four serotypes, DEN-1, DEN-2, DEN-3, and DEN-4 (Jawetz, E., Melnick, JL., &Adelberg, 2016). It is transmitted by the bite of an *Aedes* mosquito infected with dengue virus (CDC, 2012). Dengue virus transmission is only through the bite of female *Aedes* mosquitos (Greenwood D, Slack R., Barer M, Irving, 2012). *Aedes* mosquitos prefer to lay eggs in artificial water containers, to live in closer to humans and to feed on human rather than other vertebrates with high preference to the urban environment (Murray, Rosenthal, 2016; Haryanto, 2018)

Dengue virus infection is one of the most contagious arboviral infections with a massive burden consequent public health in more than 100 tropical and subtropical countries in South East Asia, Western Pacific, and South America (WHO, 2011). In Indonesia, during 2016, the IR of dengue cases was high up to 77.96 for every 100,000 populations in each province with the Case Fatality Rate (CFR) was 0.79% (Kemenkes RI, 2016). The trend of dengue virus infection in Indonesia has changed from children to older groups (Karyanti et al., 2014). An adult patient has the possibility of more severe clinical manifestations like headaches, bone pain, depression, and pathetic (WHO Media Centre, 2017).

Aedes aegypti is commonly found in a particular location such as water disposal or water storage. Exposure to such an environment may be related to specific demographic factors such as gender (Aamir et al., 2014). Gender and sex differences may contribute to the differences in the pathogenesis of infectious disease in males and females. Analysis and studies in Singapore, Vietnam, and Malaysia found that female gender was associated with severe dengue (Huy et al., 2013). However, a study in France and Lahore showed that the male gender was associated with severe dengue manifestations (Aamir et al., 2014). Both sexes that refers to the genetic and biological status of XX or XY organism and gender that refers to the social and cultural differences play together in immunological dimorphism. Females typically develop higher innate immunity, humoral, and cellular response to viral infections than males (Ruggieri et al., 2016 ; Jones et al., 2018), while testosterone appears to inhibit IgM and IgG production (Kanda, Tsuchida, & Medicine, 1996). In addition to biological differences, gender refers to the differences between male and female regulated by cultural and social factors, involving the area of human life. In developing countrywoman spends more time at home than male, thus are more exposed to indoor pollution. Inadequate nutrition and less accessibility to appropriate health care service are also more frequent in the woman (Ruggieri et al., 2016). These differences could play in discrepancies between males and males in response to infection.

As gender roles and the exposure change over the human life span, it is crucial to know the different progression of DHF between male and female adult patients by observing laboratory parameters such as thrombocyte. Daily thrombocyte count was used because it is an important laboratory parameter to help identify patients at the high-risk condition of Dengue Shocked Syndrome (DSS) (Lam et al., 2017). The pattern of thrombocyte count in both male and female patients will give new information about the different progression of the disease in both groups. This data is also important to improve the alertness to the patient's condition.

METHODS

This is an analytic observational study with a retrospective design using the secondary data collected from the medical records in Dr.Soetomo General Hospital Surabaya during 2017-2018. The sample used was adult patients

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in the age range of 18-55 years old with Dengue Haemorrhagic Fever (DHF) grade I until III, hospitalized at the tropical ward, Dr.Soetomo hospital, Surabaya. The patients included in this study did not have comorbid or coinfection that might interfere with the thrombocyte count. They were a healthy person before or having no other disease during hospitalized. Sysmex XN-1000 Hematology Analyzer analyzed the thrombocyte count. The blood samples result on the 3rd until the 10th day of illness were taken for this study. The sampling technique was total sampling and obtained 40 samples. The collected data was presented in tables, graphics, and narratives. Statistical analysis used descriptive calculations and analytical calculations (Chi-square and independent t-test). A 'p' value less than 0.05 was taken to be statistically significant. This study has passed the ethical clearance number 0500/KEPK/VIII/2018 from Dr. Soetomo Hospital.

RESULTS

Patients Characteristics

The total number of patients in the age range of 18-55 years old who infected with Dengue virus was 122 patients. Of them, 40 patients showed a clinical manifestation of Dengue Hemorrhagic Fever (DHF), and 22 patients were Dengue Fever (DF). Sixty out of 122 patients were excluded from this research because there were underlying diseases such as typhoid fever, myocarditis, urinary tract infections, varicella, hepatitis, and leukemia accompanying dengue infection. There were 16 (40%) female patients and 24 (60%) male patients. Among a total of 16 (40%) female patients with DHF, 5(12.5%) were DHF grade I, 10 (25%) were DHF grade II, and 1(2.5%) was DHF grade III. Of them,14 (35%) were at 18-35 years old, and 2(5%) were at 36-55 years old. From eleven (27.5%) out of 24 (60%), male patients were DHF grade I, 12(30%) were DHF grade II, and 1 (2.5%) was DHF grade III **(Table 1).** Of them, 20 were at 18-35 years old, and four patients were at 36-55 years old. The results of the Chi-Square statistical analysis showed that there was no significant difference between the number of male and female patients with p>0.05 (0.206) **(Table 2).** This result indicated that DHF was not affected by gender; either male or female could undergo DHF.

Table 1. Patients characteristic according to gender and clinical manifestation

Gender	Clinical Manifestation			Total
	DHF I	DHF II	DHF III	
Female	5 (12.5%)	10(25%)	1(2.5%)	16(40%)
Male	11(27.5%)	12(30%)	1(2.5%)	24(60%)
Total	16(40%)	22(55%)	2(5%)	40(100%)

Table 2. Distribution of DHF patient according to gender and age range

Gender	Number of patients by age range		Total	p
	18 – 35 years old	36 – 55 years old		
Female	14(35%)	2(5 %)	16(40%)	0.206
Male	20(50%)	4(10%)	24(60%)	
Total	34(85%)	6(15%)	40(100%)	

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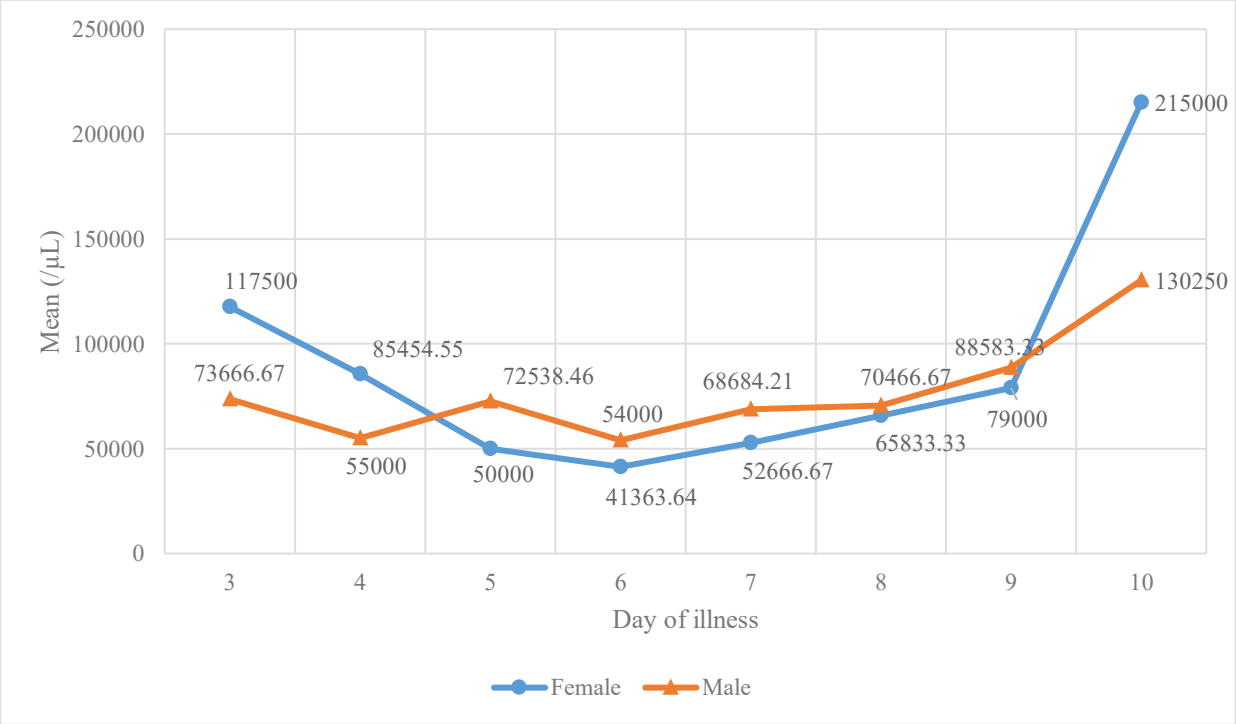

Graphic 1.Thrombocyte count pattern in DHF patient according to the day of illness

Table 3. The difference of thrombocyte count between male and female patient according to the day of illness

	Female			Male			p
Day of illness	n	Mean(/µL)	SD	n	Mean(/µL)	SD	
3	4	117,500.00	57,645.468	3	73,666.67	62,819.848	
4	11	85,454.55	44,824.912	9	55,000.00	38,421.999	0.125
5	10	50,000.00	34,140.234	13	72,538.46	49,304.522	0.231
6	11	41,363.64	29,513.633	18	54,000.00	39,615.802	0.370
7	9	52,666.67	39,764.934	19	68,684.21	59,091.316	0.623
8	6	65,833.33	31,801.992	15	70,466.67	34,073.171	0.778
9	1	79,000.00		12	88,583.33	44,935.223	
10	1	215,000.00		8	130,250.00	73,976.348	

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DISCUSSION

Sample Characteristic

Dengue virus infection remains a significant challenge in Indonesian public health. This condition is supported by the fact that Indonesia has a tropical climate and subsequent relative high humidity. It makes a favorable condition for vector-borne disease transmission, such as dengue. An epidemiology study reported that the incidence of DHF over the past 45 years increased rapidly, with the peak incidence changing from young children to 15 years old or more. The number of males was higher than females in both age ranges, but there was no significant difference between them ($p = 0.206$). Of the 40 patients, 85% were in the age range, 18-35 years old (Karyanti et al., 2014). This result was concordance with the studies carried out by Payyappilly, Karunakaran, & Adilat (2017); Mubarak, Alghasali, & Bahashwan (2017) and Raza et al. (2014). These consistent results reinforce some factors that might contribute to the more number of male patients suffered from DHF rather than females. It is widely known that in Asian countries and Saudi Arabia, males in reproductive age spend more time outside their houses and thus more likely to be exposed with the vector of dengue (Rathore et al., 2005; Shahina, et al., 2009). However, in Indonesia, the fewer female of DHF patients was not only due to the rarely spent their time out of the house, but also related to the increase in hijab usage.

The small number of samples in this study because of the exclusion of patients with comorbid conditions or coinfection such as typhoid fever, myocarditis, urinary tract infections, acute kidney injury, varicella, hepatitis, and leukemia. The comorbid condition in DHF patients was reported that the risk of dying of 326,380 hospitalized dengue cases in Brazil was 11-times higher in the presence of common underlying comorbidities (Werneck et al., 2018). In addition, co-infection with typhoid fever was often found in dengue infection caused by overlapping symptoms creating a diagnostic dilemma (Sharma et al., 2014). Major underlying diseases caused fatality cases of refractory shock in an elderly patient infected with dengue included hypertension, diabetes mellitus type 2, neoplasm, and chronic kidney disease (Kuo, Lee, & Liu, 2018). Those factors were considered to cause bias; therefore, patients with comorbid conditions or co-infection were excluded from this study.

Thrombocyte Count

This study found that the pattern of thrombocyte count in both male and female with DHF were similar. The average of thrombocyte was $< 100000/\mu L$ in both genders on the 4th till the 9th day of illness. The lowest level of thrombocyte in both genders was found on the sixth day or later than in the children who found earlier on the 5th day of illness (Sari, Kahar, & Puspitasari, 2017). This finding indicated that alertness has to be taken cautiously earlier in DHF pediatric patients rather than the adult.

The androgen promotes Th1 type immune response with the production of pro-inflammatory cytokines such as IL-2, IFN-γ, and enhanced cytotoxic T lymphocytes (CTL) activity (Fairweather & Frisancho-kiss, 2008). At serum level, estrogen also stimulates Th1 type immune response in males and females in the luteal phase or post-menopause, while at a high level as in pregnancy or pre-ovulatory periods, it promotes anti-inflammatory, Th2 type immune response (Payyappilly et al., 2017). These mechanisms are supposed to be the causes of the wide spreading of DENV and a greater decrease of thrombocyte count in the male patient rather than female patients at reproductive age in some studies. As thrombocytopenia is associated with clinical outcome and severity of the disease (Azeredo, Monteiro, & Pinto, 2015), it was assumed that

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the lower of thrombocyte count, the more severe the disease. However, in this study, we found that the average of thrombocyte count on 5th till 9th day in female patients was precisely lower than male patients. As we know, the critical phase in DHF usually happens on the 3rd till the 7th day of illness. Also, there were no significant differences of thrombocyte count between male and female in early, critical or convalescence phases ($p > 0,05$). These finding was similar to result reported by Acharya, Khan, Kosuru, & Mallya (2018) where there was no relation of mortality with gender of the dengue patient ($p = 0.534$).

Although some studies have explained that sex and gender difference might affect the progressivity of the disease, there was no clear explanation by which mechanism it affects the disease. The male patient might have greater exposure to the vector of dengue, but it did not mean they had worse outcome or shock condition because of its biological or gender factor. Whereas a female patient who has a better immune response to viral infection and less exposure did not mean they had a better outcome. We considered comorbid factors or coinfection as a major factor that might contribute to the possibility of the severe outcomes of the disease rather than sex or gender difference itself.

There are some limitations to our study. Our data lacked primary and secondary dengue infection data, which might interfere with the thrombocyte count in both genders. The thrombocyte count was not checked daily, and this made our sample size was inconsistent day by day. The other factor, such as the menstrual cycle and psychological condition, could not be evaluated. Thus, the following study is necessary to validate our findings.

CONCLUSION

The average of thrombocyte count in both male and female patients decreased since the 3rd day of illness and reached the lowest level on the 6th day of illness, then increased on the 7th day but did not reach the normal range. Although the majority of thrombocyte count in females was lower than males, there was no significant difference in thrombocyte count between them.

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Literature Review

Electrocardiogram abnormality and distance covered during six-minute walk test on type 2 Diabetes Mellitus

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Abstract

Diabetes Mellitus (DM) is a metabolic disease that has the characteristics of hyperglycemia that occurs due to abnormalities of insulin secretion, insulin action, or both. Macrovascular complications are often also referred to as secondary atherosclerosis due to DM, which can cause cerebral vascular disease, coronary arterial disease, and peripheral atrial disease. Diagnostic tools are needed for early detection in cardiovascular diseases which is accurate, inexpensive, comfortable, and available in almost health center. One of the diagnostic tools for early detection of cardiovascular diseases is an electrocardiogram (ECG). Six Minute Walk Test (SMWT) is a simple, objective, inexpensive, and efficient test to assess functional capacity and prognosis. This study aims to see whether there is a correlation between abnormalities of ECG and distance covered during SMWT in DM patients. This study was a cross-sectional study design from DM patients in the Polyclinic of the Muhammadiyah Hospital in Palembang. Forty patients with type 2 DM who fulfilled inclusion and exclusion criteria were selected using a consecutive sampling method. Twenty-seven patients have abnormal ECG, and only thirteen patients had distance covered during SMWT >300m. There is a correlation between abnormalities of ECG and distance covered during SMWT in DM patients with a significance value p = 0.011.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease that has the characteristics of hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action, or both (Purnama, 2014). This is a chronic disease where long-term treatment is needed in addition to ensuring an adequate supply of glucose to the tissues, control, and protection from damage caused by hyperglycemia are also needed. The state of hyperglycemia can cause direct and indirect effects. Generally, this damage is divided into microvascular (microangiopathy) and macrovascular (macroangiopathy) complications (Fowler, 2011). Macrovascular complications are often also referred to as secondary atherosclerosis due to DM which can cause Cerebral Vascular Disease (CVD), Coronary Atrial Disease (CAD), Peripheral Atrial Disease (PAD) and other vascular diseases (Katakami, 2018).

One of the most common causes of mortality in people with DM is cardiovascular disease (CVD) (da Rocha Fernandes et al., 2016). According to WHO, in 2008, deaths due to CVD in Indonesia reached 400 per 100,000 people in men, and 300 per 100,000 people in women, and that number continues to increase. Early diagnosis tool on CVD is needed, which is accurate, fast, and comfortable for patients, available in various health service centers in Indonesia and easily accessed. One of the diagnostic tools in CVD is the Electrocardiogram (ECG) (Qureshi, Tabinda, & Vehra, 2017). In macrovascular complications of DM, there is a lack of oxygen supply in heart muscle cells due to decreased blood flow. It can decrease the energy formation, then disruption of ion exchange for depolarization and repolarization. All of this process leads to disruption of heart muscle contraction. The process of depolarization and repolarization will be recorded on ECG records; therefore, ECG plays a role in evaluating and providing information about the status of the heart (Hampton, 2013).

Six Minute Walk Test (SMWT) is a simple, objective, inexpensive, and efficient test for assessing functional capacity and the prognosis of patients (Adiniyi, Uluko, & Sani-Sulaiman, 2009). This test can reflect daily activities and can evaluate all systems involved. Low performance at SMWT can reflect poorer clinical outcomes, such as impaired vascular reactivity, higher risk of systemic heart disease, and describes a low level of physical activity among patients.

The importance of ECG images in detecting CVD in DM patients is associated with a decrease in functional capacity due to CVD that has an impact on the distance covered during SMWT. Therefore, an analysis of the relationship between ECG abnormalities and the distance covered during SMWT in type 2 DM patients is needed.

METHODS

This observational analytic study with a cross-sectional study design conducted at the Palembang Muhammadiyah Hospital Polyclinic in October 2018 involved 40 patients who met the inclusion criteria. Range of age between 40 to 70 years old and had been diagnosed with type 2 DM. The exclusion criteria were not willing to take part in the study, neuromusculoskeletal disorders in the inferior limb (trauma, ulcer, arthritis, hemiparesis, paralysis, and others), unstable angina or myocardial infarction within in a month, systolic blood pressure> 180 mmHg and diastolic> 100 mmHg, cognitive impairment, pregnant patients, CHF NYHA>III and moderate to severe asthma. Samples were selected using the consecutive sampling method.

Research data were collected from type 2 DM patients who met the inclusion criteria by giving informed consent and interviews to find out additional information needed for the

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study. Then, the patient performed ECG and SMWT. After the data have been collected, a correlation test is performed between ECG abnormalities and distance covered during the SMWT using the Chi-Square test.

RESULTS

Forty patients aged 40-70 years old fulfilled the inclusion and exclusion criteria. Twenty-seven respondents had an abnormal ECG, and only 13 had distance covered during the SMWT above 300 meters. Abnormal ECGs found in this study are left ventricular hypertrophy, left atrial hypertrophy, left and right axis deviation, right and left bundle branch block, old myocardial infarction, ST-segment depression, T inversion, and atrial fibrillation.

Table 2 shows that 22 respondents who have abnormal ECG have distance on the SMWT ≤300 meters, and 8 out of 13 respondents with normal ECG have distance covered >300 meters on the SMWT. There is a relationship between ECG abnormalities and the distance covered during SMWT with p = 0.011 (p <0.05).

Table 1. Baseline Characteristics

Characteristic	N	(%)
Age		
40-49 years old	7	17,5
50-59 years old	17	42,5
60-70 years old	16	40,0
Sex		
Man	16	40,0
Woman	24	60,0
Nutritional Status		
Underweight	2	5,0
Normal	19	47,5
Overweight	7	17,5
Obesity	12	30.0
Smoking Status		
No	29	72,5
Yes	11	27,5
6MWT Distance		
≤300m	27	67,5
>300-400m	13	32,5
ECG		
Normal	13	32,5
Abnormal	27	67,5

Table 2. Comparison of ECG and Distance Covered during SMWT

			SMWT		Total	
			≤300 meter	>300 meter		
ECG	Abnormal	Count	22	5	27	p=0,011
		Expected Count	18,2	8,8	27,0	
	Normal	Count	5	8	13	
		Expected Count	8,8	4,2	13,0	
Total		Count	27	13	40	
		Expected Count	27,0	13,0	40,0	

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DISCUSSION

In this study, the results show that 27 of the 40 respondents had abnormal ECGs. The result was consistent with the opinion of Papa et al. (2013), where cardiovascular complications are a major cause of disability and death in patients with type 2 DM, and the risk of cardiovascular disease (CVD) is two to eight times higher in diabetic patients compared with non-diabetic patients of the same age, sex, and race (Papa et al., 2013).

Diabetes is associated with the development of a premature cardiovascular disease, which relates to the clustering of risk factors such as dyslipidemia, hypertension, obesity, and hyperglycemia in the presence of insulin resistance. In addition, diabetes is associated with an inflammatory and pro-thrombotic environment, exacerbating the development of atherothrombosis. Insulin resistance and hyperglycemia both contribute to the development of endothelial cell dysfunction and increased oxidative stress, culminating in accelerated atherosclerosis. Clot formation and function are also directly affected by insulin resistance and hyperglycemia, with increased levels of coagulation factors and anti-fibrinolytic proteins and a fibrin network that is more resistant to lysis, coupled with increased platelet activation (King & Grant, 2016).

There are many theories and hypotheses about the relationship between type 2 DM and cardiovascular disease. Three-quarters of the five million deaths due to DM are related to CVD as a complication. The ECG as an early diagnosis for CVD in type 2 DM becomes very important. In research at the Endocrine Outpatient Clinic of BLU Prof. Dr. dr. R. D. Kandou Manado, found LAH picture, coronary artery disorders (myocardial ischemia and old myocardial infarction), blockade of the bundle branches, and left ventricular hypertrophy in DM patients (Maradjabessy, Rampengan, & Langi, 2015).

Cardiovascular complications in people with DM are related to changes in ECG. Several studies have shown a relationship between DM and left ventricle hypertrophy (LVH). The Strong Heart Study (SHS) reports an increase in left ventricular mass and ventricular wall thickness in both women and men with DM. Similar findings were reported in The Cardiovascular Heart Study (CHS) and The Multi-Ethnic Study of Atherosclerosis (MESA). A recent study in type 2 DM patients in Japan reported an association between insulin resistance, arterial stiffness, and left ventricular mass index (using cardiac MRI). This finding is also supported by studies with a more significant number of samples in Sweden that show an association between metabolic syndrome, insulin resistance, and increased mass and thickness of the ventricular wall (Shahab, 2014).

Other ECG features found in DM patients are prolongation of the QT interval and QT dispersion, even at the onset of DM (Stern & Sclarowsky, 2009). In a review of an article by Movahed MR mentioned that there are several non-randomized studies that report an increase in the prevalence of heart conduction blockade in DM patients, such as right bundle branch block (RBBB), bifascicular block and high degree atrioventricular (AV) block (Movahed, 2008).

The result of this study shows there is an association between abnormal ECG and distance covered during SMWT. Cardiovascular complications in DM patients that can be detected on the ECG are associated with decreased functional capacity linear with research by Ramos et al. (2015) found that reduced distanced covered in SMWT occurs due to manifestations of diabetes mellitus. It can occur because of a lack of activity that leads to decreased function, or conversely, a decrease in

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glucose tolerance will lead to cardiopulmonary complications (Ramos et al., 2015).

A similar study conducted by Adiniyi, Uluko, and Sani-Sulaiman (2009) found that DM patients had lower SMWT values than non-DM patients, significantly lower in women, old age, and obese patients. It is likely because DM patients are closely related to cardiovascular and pulmonary disorders, which have been shown to significantly reduce functional capacity (Adiniyi et al., 2009).

CONCLUSION

There is a relationship between ECG abnormalities and distance covered during SMWT in patients with type 2 DM with a significance value of p = 0.011 (p <0.05).

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Literature Review

In vitro test: antimicrobial activity potential from Ciplukan fruit (*Physalis minima* L.) extract in *Methicillin-resistant Staphylococcus aureus* (MRSA)

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Abstract

In Indonesia, in 2006, the prevalence of infections due to MRSA was 23.5%. *Physalis minima* L. plants are known to have antimicrobial activity because they contain compounds withaferin A, which can induce programmed cell death. This research was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Ciplukan (*Physalis minima* L.) extract in Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria. Dilution test with Mueller-Hinton broth medium used for measuring the minimum inhibitory concentration (MIC). Ciplukan fruit extract was dissolved in distilled water, and poured into a test tube with a certain concentration (0.9 g/mL (90%); 0.3 g/mL (45%); 0.15 g/mL (22.5%); 0.075 g/mL (11.25%) and 0.0375 g/mL (5.625%). After being incubated for 24 hours, the bacteria in the test tube were plated on nutrient agar plates to determine the MBC. The MIC cannot be determined, because the medium in the dilution test tube is disturbed by the color of the extract so that turbidity cannot be observed. From the observations of the minimum bactericidal concentration, MBC of the Ciplukan (*Physalis minima* L.) fruit extract against MRSA was in the P1 tube or equivalent to 0.9 g / ml (90%).

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INTRODUCTION

The carelessly use of antibiotics against the *Staphylococcus aureus* can cause resistance, such as *Methicillin-resistant Staphylococcus aureus* (MRSA). From 2004 to 2006, around 77% of nosocomial infections in Korea were caused by MRSA, as well as in Taiwan as many as 55% (Chen & Huang, 2014). As many as 29%-35% of *Staphylococcus aureus* isolates in all hospitals in America, and Europe has MRSA strains (Haddadin et al., 2013). In Indonesia, in 2006, the prevalence was 23.5% (Sulistiyaningsih, 2010). Conducted from Antimicrobial Resistance (AMR), surveillance showed that from a total 250 *Staphylococcus aureus* isolated found that 45 (18%) of them were are MRSA (Hadi et al., 2013).

The MRSA has beta-lactamase, which makes the MRSA have resistance to all beta-lactam antibiotics, which means that antibiotics that have beta-lactam rings cannot be used to treat infections due to MRSA (Rasigade & Vandenesch, 2013). The MRSA is also known to have the mecA gene and fem gene. *Staphylococcus aureus* is also often found to have additional resistance mechanisms from plasmids, prophages, and transposons that have the potential to spread MRSA strains to other *Staphylococcus aureus* bacteria (Lowy, 1998).

Until now, there has been no research on the potential for Ciplukan (*Physalis minima* L.) as an antibacterial agent such as MRSA. It is necessary to test the antimicrobial potential of the Ciplukan (*Physalis minima* L.) plant against MRSA.

METHODS

Preparation of Plant Extract

The sample used was Ciplukan fruit (*Physalis minima* L.) obtained from the Gresik area. Samples that have been collected will be identified in the Materia Medica Batu to find out the type and species of the sample.

Samples obtained from Gresik areas were collected and made in the form of Simplicia. Simplicia obtained as much as 300g. Simplicia will be soaked with 70% alcohol as much as 750 ml liters in the tube for 3 x 24 hours. The filtrate obtained from the immersion will be concentrated with a rotary evaporator. The extract obtained was 180mg. The extract is stored at 4 ° C.

Ethical Clearance

This research was carried out based on a certificate of ethics made by the Health Research Ethics Committee of the Faculty of Medicine, Airlangga University, Surabaya, with certificate number No.141 / EC / KEPK / FKUA / 2019.

Preparation of Bacterial Specimen

The MRSA bacterial specimens were obtained from bacterial storage in the Department of Microbiology, Faculty of Medicine, Airlangga University.

Inoculation Preparation

The existing stock is cultured in advance so that healthy growth is obtained (it thrives and in the logarithmic growth phase or does not experience mutations or lag or die phase). The bacterial colony is taken using a loop and transferred to a tube that was previously filled with nutrient broth. Bacteria were incubated for 24 hours at 37ºC. The turbidity proportion is equivalent to 0.5 *McFarland*.

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Preparation of Antimicrobial Test

Antimicrobial activity tests were carried out by the dilution method in the Mueller-Hinton media. Ciplukan (*Physalis minima* L.) fruit extract was diluted with distilled water (*aquadest).* Then, Ciplukan fruit extracts divided into five test tubes with different concentrations. P1 tube 90% (g/mL), P2 45% (g/mL) tube, P3 tube 22.5% (g/mL), P4 tube 11.25% (g/mL), and P5 tube 5.625% (g/mL). Each tube will be given 1 mL of bacterial suspension except the control tube. Repetition is done five times.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits bacterial growth after being incubated for 24 hours at 37 ° C.

The minimum bactericidal concentration (MBC) is the concentration of an antimicrobial agent needed to kill certain bacteria. The minimum bactericidal concentration can be determined from the antimicrobial agent, which has been determined the minimum inhibitory concentration after incubation for 24 hours at 37 ° C.

RESULTS

Observations on bacterial growth are made through two methods: 1) Dilution test to determine the MIC; 2) cultured bacteria on nutrient agar plates to determine the MBC.

Dilution test was carried out using seven tubes, including positive and negative control tubes. Each tube was filled with extract concentrations of 90% (g / mL), 45% (g / mL), 22.5% (g / mL), 11.25% (g / mL), and 5.625% (g / mL). For positive control containing MRSA colonies and negative control containing 90% (g / mL) extract without MRSA.

Table 1. Observation of MIC in Bacterial Dilution Test

Tube	Concentration	Observation of the Medium Color in the Tube				
		Replication				
		1	2	3	4	5
K 1	90% (g/mL) extract	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple
K 2	MRSA	Yellow	Yellow	Yellow	Yellow	Yellow
P 1	90% (g/mL) extract + MRSA	Dark Purple	Dark Purple	Dark Purple	Dark Purple	Dark Purple
P 2	45% (g/mL) extract + MRSA	Dark Brown	Dark Brown	Dark Brown	Dark Brown	Dark Brown
P 3	22,5% (g/mL) extract + MRSA	Brown	Brown	Brown	Brown	Brown
P 4	11,25% (g/mL) extract + MRSA	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown
P 5	5,625% (g/mL) extract + MRSA	Dark Yellow	Dark Yellow	Dark Yellow	Dark Yellow	Dark Yellow

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Table 2. Observation of Bacterial Growth in Agar Plate Nutrient

Tube	Concentration	Observation of Bacterial Growth in Agar Plate Nutrient				
		Replication				
		1	2	3	4	5
K 1	90% (g/mL) extract	-	-	-	-	-
K 2	MRSA	+	+	+	+	+
P 1	90 % (g/mL) extract + MRSA	-	-	-	-	-
P 2	45 % (g/mL) extract + MRSA	-	+	+	+	-
P 3	22,5 % (g/mL) extract + MRSA	-	+	+	+	-
P 4	11,25 % (g/mL) extract + MRSA	+	+	+	+	+
P 5	5,625 % (g/mL) extract + MRSA	+	+	+	+	+

'+' show bacterial growth, while '-' no bacterial growth
MRSA : Methicillin-resistant *Staphylococcus aureus*

Observation of dilution results found that each tube has a different color. The difference in color was due to differences in concentration between the tubes. Dilution test results are carried out visually by comparing the level of turbidity between the treatment tube and the control tube. In Table 1. we can see the colored medium in the tube so that the MIC cannot be determined. The medium color is determined subjectively.

Then Each tube will be cultured on nutrient agar plates to determine the MBC. The result of the MBC showed in Table 2. In the first and fifth replications, minimum bactericidal concentration was observed at a concentration of 22.5% (g/mL), and at the second to fourth replication, the MBC was observed at a concentration of 90% (g/mL). In the positive control, found bacterial growth on nutrient agar plates and in the negative control tube found no bacterial growth. The absence of bacterial growth in the negative control also indicated the extract was not contaminated.

DISCUSSION

Antibacterial Mechanism of Ciplukan Fruit Extract

Methicillin-resistant *Staphylococcus* damages the structure of β-Lactam antimicrobials by producing beta-lactamase and has structural changes in the binding protein penicillin. MRSA strains have two resistance mechanisms, namely intrinsic (innate) and extrinsic (acquired). Intrinsic resistance will continue to be attached to bacteria and never change. The intrinsic mechanism of MRSA is the presence of the mecA gene that encodes PBP2a. In addition to the mecA gene, MRSA also has a fem gene. Fem gene, which can make MRSA has resistant to methicillin, penicillinase-resistant penicillins, and cephalosporin (Jansen et al., 2006).

Staphylococcus aureus peptidoglycan has a long characteristic in the form of a woven structure (cross-linkage) with a flexible pentaglycine side chain. This peptidoglycan is an antimicrobial target of β-lactam. Resistance occurs when there is a change of structure in PBP2a because of the production of the beta-lactamase enzyme that can catalyze the β-Lactam antimicrobial so that the antimicrobial β-Lactam cannot damage

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the structure of peptidoglycan. Penicillin-binding protein is a group of proteins involved in peptidoglycan biosynthesis, which catalyzes the transpeptidation reaction (Memmi et al., 2008). The transpeptidation reaction by PBP is required at the time of bacterial cell wall synthesis. Penicillin-binding protein is also a target of the β-Lactam protein so that if there is a change in the structure of PBP, the β-Lactam protein will be inactive and cannot interfere with cell wall synthesis. Some of the mechanisms that cause acquired resistance are efflux pumps, impermeability mutations, carbapenemases, and aminoglycoside-modifying enzymes.

The active compounds in Ciplukan are within one, withanolide A, withaferin A, dihydroxyphysalin B2-4, and physalin A (Chothani & Vaghasiya, 2012). Withaferin A is a steroidal lactone isolated from a medicinal plant from India, *Withania somnifera*. Withaferin A is widely known as an anti-inflammatory, anticancer and antimicrobial. Withaferin A can inhibit cell growth and induce apoptosis (Sail & Hadden, 2012).

The mechanism of action of withaferin A is to bind the metabolic enzymes that have amino acids with the SH group. Amino acids that have an SH group are methionine and cysteine. Methionine is an essential amino acid containing sulfur. Methionine is the precursor of any metabolite protein such as succinyl-CoA, homocysteine, cysteine, creatine, and carnitine. Methionine also helps the biosynthesis of glutathione, which functions to fight oxidative stress on cells (Martínez et al., 2017). The withaferin A bond to the SH group that will inactivate the metabolic enzymes, induce oxidative stress because glutathione is not formed and cut the glycolysis pathway because it destroys succinyl-CoA so the cells will start to die.

The content of the active substance Withaferin A as an antibacterial is also aided by the action of flavonoids, which are lipophilic substances which can damage bacterial cell membranes that will cause the release of metabolites from the cell and the entry of fluid from the outside into the cell. Alkaloids are heterocyclic nitrogen compounds that are known to be able to interfere with nucleic acid production and cell division (Cushnie et al., 2014; Narasimha Rao & Raman Venkatachalam, 1999)

Minimum Bactericidal Concentration (MBC) Determination

Table 3. Antimicrobial Activity of Ciplukan Extract From any Studies

Microba	MBC	Reference	Part of the Plant
B. Subtilis, E.coli, P. solanacearum	2mg/mL	(Shariff et al., 2006)	Leaf
X. Vesicatoria	4mg/mL	(Shariff et al., 2006)	Leaf
B. subtilis, P. solanacearum, and *X. Axonopodis*	2mg/mL	(Shariff et al., 2006)	Callus
E.coli and *X. vesicatoria*	4mg/mL	(Shariff et al., 2006)	Callus
Methicillin-resistant *Staphylococcus aureus*	70% (ml/ml)	(Fitrianti et al., 2011)	Leaf
Methicillin-resistant *Staphylococcus aureus*	90% (g/mL)	Author's	Fruit

MBC: Minimum Bactericidal Concentration

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Minimum Bactericidal Concentration (MBC) Determination

From table 3, the research has varying results from the first replication to replication five. In the first replication, MBC was observed in P3 tube (22.5% (g / mL)). But in the second replication, MBC can be observed in P1 tube (90% (g / mL)), third replication in P1 tube (90% (g / mL)), fourth replication in P1 tube (90% (g / mL)), and the fifth replication, MBC can be observed in P3 (22.5% (g / mL)). This study was compared with previous research on antimicrobial activity testing of Ciplukan (*Physalis minima* L.) fruit extracts.

Ciplukan (*Physalis minima* L.) leaf extracts have antimicrobial mechanism against *B. Subtilis, E.coli, P. solanacearum,* and *X. axonopodis* at a concentration of 2mg/mL while *X. Vesicatoria* at a concentration of 4mg/mL. Also, Ciplukan (*Physalis minima* L.) callus extracts also showed the minimum bactericidal concentration of *B. subtilis, P. solanacearum,* and *X. axonopodis* at a concentration of 2mg/mL whereas in *E.coli* and *X. vesicatoria* at a concentration of 4mg/mL (Shariff et al., 2006).

A similar study conducted with *Physalis angulata* L. leaf extract found that at a concentration of 70% could kill (MBC) MRSA bacteria (Fitrianti et al., 2011).

In this study of Ciplukan fruit extract against the MRSA bacteria, the bacteria could not grow on the media at a concentration of 22.5% (g / mL) in the first and fifth replications, but in the second, third and fourth replication bacteria could not grow at a concentration of 90% (g/mL).

Other studies have also shown similar results, namely differences in the rates of MBC in replication. The differences in the MBC ethanol extract of *Zingiber cassumunar Roxb*, between concentrations of 12.5%; 25%; and 50%. At a concentration of 50% already, no bacterial growth was found in all replications, a concentration of 25% obtained bacterial growth in 1 of 7 replications, a concentration of 12.5% was obtained growth in 4 of 7 replications. The MBC is determined at the smallest concentration where there is no significant difference compared with negative controls, and there are significant differences when compared with positive controls so that the MBC is determined at a concentration of 25% (Raharjoyo & Gunardi, 2009). From that, we conclude that the MBC in this study was 90% (g/mL).

This study could not determine the MIC because the solution in the Ciplukan (*Physalis minima* L.) fruit extract was colored, so the turbidity level to determine the MIC was not possible. Therefore, it is necessary to filtrate the solution to make it colorless. Another way is to make an extract with another method so that the extract will be colorless. Further research in vivo is needed to determine the dosage and side effects of the Ciplukan fruit.

CONCLUSION

Ciplukan fruit extract (*Physalis minima* L.) has an antibacterial effect against MRSA. The minimum bactericidal concentration (MBC) in this study was 90% g/mL. The MIC cannot be determined.

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Research Article

Pneumonia degree correlation in children with clean and healthy behavior (CHB)

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Abstract

Pneumonia is an acute infection that affects the lung with symptoms of cough, shortness of breath, grunting, cyanosis, rhonchi, and infiltrates in thorax imaging. Based on The Integrated Management of Sick Children in handling Acute Respiratory Tract Infection program, pneumonia is classified as severe pneumonia, pneumonia and not pneumonia. One of the risk factors of pneumonia is the low level of Clean and Healthy Behavior (CHB). The purpose of this study was to determine the degree of pneumonia in children with clean and healthy behavior (CHB). The analytic observational study with cross-sectional method. The sample was conducted children under five years old diagnosed with pneumonia in the Children's Ward of Rachma Husada Hospital Bantul with due regard to inclusion and exclusion criteria. Sampling was carried out using consecutive techniques. The data was retrieved using secondary data (medical records) and observing CHB with questionnaire. The data was analyzed using fisher's exact test. From 32 samples in this study, the majority of samples suffered from severe pneumonia (81.3%) and the most CHB indicators were the Intermediate category (50%). The result of bivariate analysis shows there was a correlation between the degree of pneumonia in children and the family's CHB (p=0.01). There was a correlation between the degree of pneumonia in children and the clean and healthy behavior (CHB).

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INTRODUCTION

Pneumonia is the leading cause of death in children under five years old. World Health Organization (WHO) estimates that the incidence of pneumonia in children under five years old in developing countries is 0.29 episodes per child each year or 151.8 million cases of pneumonia each year, of which 8.7% or 13.1 million cases include severe pneumonia and need to be hospitalized. There are 15 countries with predictions of new cases and the highest incidence of pneumonia in children under five years old, covering 74% or 115.3 million cases out of 156 million cases worldwide. More than half are focused in 6 countries, namely India, China, Pakistan, Bangladesh, Indonesia, and Nigeria, where up to 44% of the population of children under five years old in the world (Rudan, Boschi-Pinto, Biloglav, Mulholland, & Campbell, 2008). The results of the 2013 Basic Health Research (Riskesdas), based on the age group of the population, the highest prevalence of pneumonia is 1-4 years of age (Kementrian Kesehatan RI, 2018).

Pneumonia is an acute infection of the lung tissue with symptoms of fever, coughing, rapid breathing, wheezing (grunting), nasal lobe breathing, retraction, cyanosis, soft wet crackles loud on auscultation, and infiltrates on lung X-rays. Following Integrated Management of Sick Children in the prevention of acute respiratory infections (ARI), pneumonia is classified as severe pneumonia, pneumonia and not pneumonia (Eka Cahyani & Anggrainingsih, 2016; WHO, 2014).

Clean and healthy behavior (CHB) is the main capital for the eradication of ARI, which can be assessed by indicators of household members behaving clean and healthy life. CHB classification is based on accumulated scores, namely, (1) primary category, (2) intermediate category, (3) *purnama* category, (4) independent category. One of the risk factors for pneumonia in children is low CHB (Kementerian Kesehatan RI, 2011; Sulistyowati, 2010).

The purpose of this study was to determine the degree of pneumonia in children under five years old with clean and healthy behavior (CHB).

METHODS

The study was conducted at the Rachma Husada Hospital in Bantul in July to August 2019. This type of research is analytic observational with a cross-sectional approach. Based on the calculation of the sample, 32 samples of children under five years old who met the inclusion and exclusion criteria were needed. Inclusion criteria include: (1) diagnosed pneumonia as assessed by history taking and supporting examination (chest radiograph), (2) having a KMS book, (3) being less than five years old. Exclusion criteria include: (1) having other diseases (asthma, bronchitis), (2) not being willing to be a respondent

Data obtained from medical records and observations using a questionnaire. The questionnaire was filled in by the respondent's parents, and the researcher directly observed the respondent's home environment. The questionnaire was taken from a guide to clean and healthy life behavior (CHB). The study was conducted after obtaining a research permit and Ethical Clearance from the medical research ethics committee at the Muhammadiyah Semarang University and after obtaining the respondent's approval to take part in the study. Data is processed using the SPSS21.0 for Windows program. Data analysis was using the *chi-square test* and *Fisher's exact.*

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RESULTS

This research was conducted from July to August 2019 in 32 children under five years old who had fulfilled the inclusion criteria. The data was taken from the medical records at The Rachma Husada Hospital Bantul and then the children's parents filled out a questionnaire related to CHB.

Based on Table 1, the results show that the majority of the age of respondents in this study between the ages of 2 to 4 years with each number is 10 respondents with a percentage of 31.2%. In the sex indicator, it was found that 19 children had male sex (59.4%) and 13 children had female sex (40.6%).

Table 2 shows the degree of pneumonia in the majority of samples was severe pneumonia (81.3%).

From table 3, the indicators for families who are at risk of pneumonia are the study samples, the majority of which do not provide exclusive breastfeeding, do not wash hands with soap, do not use healthy latrines, do not eradicate mosquito larvae, do not eat fruits and vegetables every day, and smoke in the house. Table 4 shows that the category of CHB in the largest sample is the Intermediate category (50.0%),

Table 1. Characteristics of Respondents

No	Indicators		Frequency	(%)
1	Age	1 y.o	1	3,1
		2 y.o	10	31,2
		3 y.o	10	31,2
		4 y.o	10	31,2
		5 y.o	1	3,1
2	Sex	Male	19	59,4
		Female	13	40,6

Table 2. Distribution of degree of pneumonia in children under five years old

No	Pneumonia	Frequency	(%)
1	Pneumonia	6	18,8
2	Severe Pneumonia	26	81,3

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Table 3. Characteristics of CHB indicators (Kementerian Kesehatan RI, 2011)

No	Indicators	samples (N=32)			
		Yes	%	No	%
1	Labor is assisted by medical personnel	32	100	0	0,0
2	Give exclusive breastfeeding	14	43,8	18	56,3
3	Weigh every month	18	56,3	14	43,8
4	Use clean water	16	50,0	16	50,0
5	Washing hand	14	43,8	18	56,3
6	Use a healthy latrine	10	31,3	22	68,8
7	Eradicate mosquito larvae	6	18,8	26	81,3
8	Eat vegetables and fruit every day	11	34,4	21	65,5
9	Physical activity	16	50,0	16	50,0
10	Smoking in the house	25	78,1	7	21,9

Table 4. Frequency Distribution of CHB categories

CHB	Frequency	(%)
Primary	1	3,1
Intermediate	16	50,0
Purnama	12	37,5
Independent	3	9,4
Totals	32	100.0

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Table 5. Relationship of the degree of pneumonia with CHB

CHB	Pneumonia				Totals		P
	Pneumonia		Severe Pneumonia				
	N	%	N	%	N	%	
Primary	0	0,0	1	100	1	100	0,010£
Intermediate	0	0,0	16	100	16	100	
Purnama	4	33,3	8	66,7	12	100	
Independent	2	66,7	1	33,3	3	100	

Based on table 5, the obtained CHB sample in the Primary and Intermediate categories all suffered from severe pneumonia. In the Purnama category, samples 66.7% suffered from severe pneumonia. Whereas in CHB 66.7% of the Independent category sample suffered from pneumonia. Statistical test results showed that there was a significant relationship between the degree of pneumonia with CHB (p = 0.01).

DISCUSSION

The results of this research, it was found that the degree of pneumonia in children under five years old was significantly related to CHB (p: 0.01). This finding is in line with Sugihatono's research (p: 0.015; OR: 3.121 C195%: 1.225 - 7.957) and Domili (p: 0.05), which states that CHB is a risk factor for several diseases including pneumonia. Children who live in environments with low CHB are more susceptible to pneumonia. It is caused by imperfect immunity and a relatively narrow respiratory tract (Rachmawati, 2013; Zairinayati, Ari Udiyono, 2013).

This study found that the majority of samples suffered from severe pneumonia (81.3%). These results are in line with Idris Handriana's study which states that a low CHB has a greater proportion of pneumonia with retraction. This is due to the lack of exclusive breastfeeding in infants and respiratory air exposed to cigarette smoke. In the study, it was found that *p value* = 0.016, which means there is a relationship between CHB and the incidence of pneumonia in infants (Idris, 2018). While in this research, the cause of pneumonia was not only the lack of exclusive breastfeeding and breathing air exposed to cigarette smoke but the lack (56.3%) of maintaining cleanliness like washing hands before eating and after defecating. In addition, due to only half the study sample conducted physical activity (50.0%).

The results of this study the majority of the sample included in the Intermediate category (50.0%). The Associate category is included in the category of CHB that is lacking, so the risk of a toddler suffering from pneumonia is quite large. This is in line with Ratna Sulistyawati's research which states that toddlers who live with a low CHB have a 3.24 times greater risk of suffering from pneumonia than those who live with a good CHB with statistical test results showing there is a significant relationship between maternal CHB with the incidence of pneumonia (p: 0,0; OR: 3,24) (Rachmawati, 2013; Sulistyowati, 2010).

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In this study it was found that the majority of sample family members were smokers (78.1%). This study is in line with the research of Dinda Rachma Anggraini, who found that there was a relationship between the presence of smokers and the incidence of pneumonia in infants. Children who live in one house with smokers have a risk 3.4 times greater than children who do not live in one house with smokers. Cigarette smoke is a pollutant in the living space and will increase the risk of illness from toxic materials in infants and will cause respiratory problems, especially increasing the risk of acute respiratory infections and lung disorders (Anggiani, 2016).

Other CHB factors, such as eradication of mosquito larvae around the house, were not carried out by the majority of the study sample (81.3%). An environment that does not make efforts to eradicate mosquito larvae will trigger the arrival of mosquitoes where mosquitoes are a source of disease and host of infectious diseases. Also, the use of clean water for daily activities such as bathing, cooking, and drinking water is only carried out by half of the study sample, so that it can cause bacteria to grow (Anggiani, 2016).

In this study, the majority of mothers did not provide exclusive breastfeeding (56.3%) due to a lack of knowledge about the benefits of exclusive breastfeeding. In addition, some mothers are not diligent in bringing their children to the *posyandu* to weigh their bodies so that mothers do not know that their children are experiencing poor or even poor nutrition. One of the factors that influence the emergence of pneumonia and the severity of the disease is the children's immune system. The immune system can be influenced by several things including: nutritional status, immunization status, exclusive breastfeeding and the age of the child. The poor nutritional status will increase the susceptibility and severity of pneumonia infection. Exclusive breastfeeding can prevent pneumonia due to bacteria and viruses. While in this study the majority (65.5%) of the sample did not consume fruits and vegetables every day so that it could cause low nutritional status (Ningsih & Jonyanis, 2014).

In this study, the results were obtained that all research samples were assisted by the birth of medical staff. This is in line with Ratna Sulistyawati's research which states that all study samples were born by medical personnel (Rachmawati, 2013). Provisions for delivery must be assisted by medical personnel in accordance with government policies in the program of maintaining maternal health and reducing maternal mortality (Ningsih & Jonyanis, 2014). Labor that is not helped by medical personnel, there will likely be a large infection because the tools used to help deliver labor are not sterile, making it easier for bacteria to grow.

In this study, the majority of the study sample (68.8%) did not use healthy latrines. This is in line with Sulistyowati's research which states that not using healthy latrines can cause infections in children (Idris, 2018; Sulistyowati, 2010). This is due to the large number of bacteria that arise in latrines so that hygiene must always be considered.

CONCLUSION

This research found that the highest degree of pneumonia in children under five years old was severe pneumonia, while the classification of the majority of CHB was the Intermediate category. There is a significant relationship between the degree of pneumonia in children and CHB.

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Research Article

Collagen type I and type II expression evaluation on cartilage defect regeneration treated with Dwikora–Ferdiansyah–Lesmono–Purwati (DFLP) scaffold supplemented with adipose–derived stem cells (ASCs) or secretome: an in-vivo study

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Abstract

Cell-based therapies such as Scaffold, stem cells, and secretome, are one of the alternatives to enhance the regeneration of hyaline-like cartilage in cases of cartilage defects. This study is an in-vivo experiment using animal models, in which we apply a composite of DFLP (Dwikora-Ferdiansyah-Lesmono-Purwati) Scaffold and Adipose-Derived Stem Cells (ASCs) or Secretome to an injury model on the distal femoral trochlea of New Zealand White Rabbits. The animals were divided into four groups: (1) control (K); (2) Scaffold only (S); (3) Scaffold + ASCs (SA); (4) Scaffold + Secretome (SS). Animals were terminated in the 12th week, and an immunohistochemistry (IHC) evaluation for Collagen type I and II were done. Statistical analysis shows that collagen type I IHC between groups shows no significant difference ($p = 0.546$). Collagen type II IHC shows significant difference between groups ($p = 0,016$). The findings in this study showed that Scaffold + ASCs group and Scaffold + Secretome have better collagen type II expression compared to the control group. DFLP Scaffold composite with ASCs or Secretome shows potential for cartilage regeneration therapy by increasing type II collagen expression as in hyaline-like cartilage which may be used for regenerative therapy for cartilage defects.

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INTRODUCTION

Articular cartilage is an avascular, aneural, and alymphatic tissue. This causes problems in the treatment of cartilage defects. Initially, inflammatory cytokines IL-1 and TNF-α will appear. IL-1 will block the stimulation of proteoglycan synthesis and stimulates MMP production, TNF-α will stimulate the production of cartilage degrading enzyme and block collagen synthesis (Fritz, Janssen, Gaissmaier, Schewe, & Weise, 2008; Schmitz, DeHart, Qazi, & Shuler, 2016).

If the defect is only at the chondral level, regeneration cannot occur, if it is at the subchondral/osteochondral level, fibrocartilage regeneration will occur, this is caused by collagen type III production by the chondrocytes. The most commonly used and developed therapy is by microfracture and cellular-based therapy, as in ACI and Scaffold. Compared to the need for 2 surgeries in ACI therapy, Scaffold based therapy only needs a single event surgery (Bedi, Feeley, & Williams, 2010; Beer, Mildner, & Ankersmit, 2017; Jang, Jung, & Ju, 2017; Mancuso, Raman, Glynn, Barry, & Murphy, 2019).

An earlier study using Freeze Dried Bovine Cartilage (FDCB) composite using Mesenchymal Stem Cells and Platelet Rich Plasma in 2017 shows good results in an animal model. The FDBC scaffold is a precursor for this study's DFLP (Dwikora–Ferdiansyah–Lesmono–Purwati) Scaffold, which in an undecellularized cartilage bovine scaffold. (Dwikora Novembri Utomo, Abdul Rantam, Ferdiansyah, & Purwati, 2017) Scaffold functions to isolate the stem cells and to capture the extracellular matrix produced by the chondrocyte to increase the mechanical properties of the regenerated material (Tuan & Mauck, 2013).

The addition of ASCs or Secretome is expected to produce more hyaline-like cartilage which has more collagen type II than collagen type I expression, which is one of the characteristics of hyaline-like cartilage rather than fibrocartilage (Henderson, Lavigne, Valenzuela, & Oakes, 2007; Ulrich-Vinther, Maloney, Schwarz, Rosier, & O'Keefe, 2003). After this therapy, the result is expected to be hyaline-like cartilage.

METHODS

This study is in an in-vivo post-test only controlled animal laboratory study done at the Stem Cell Research and Development Center Laboratory. In this study a model cartilage defect of 4.5 mm^2 was made on 24 New Zealand White Rabbits (*Oryctolagus cuniculus)* which were randomly divided into four groups: (1) Control group (K); (2) Scaffold only group (S); (3) Scaffold + ASCs group (SA); (4) Scaffold + secretome group (SS). The randomization was done using physical methods by shuffling designated groups in each subject. The sample size was quantified using the degree of freedom of ANOVA (Charan & Kantharia, 2013; Ilyas, Adzim, Simbak, & Atif, 2017). The rabbits were aged 8-12 months, weighs 2400-3200 grams, and are healthy until the end of the study. Each group consists of 6 rabbits. This experiment was ethically approved by the Animal Care and Use Committee of Airlangga University Veterinary Medicine Faculty (Certificate number: 2.KE.060.04.2019). Each rabbit was placed in a cage 100 cm x 50 cm x 70 cm, free access to food (300 grams daily), and water. Before the study, the rabbits were acclimated to the conditions for 1 week. Rabbits that were deemed not healthy or failed to acclimate with the conditions within the acclimatization period were excluded from this study and replaced.

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Anesthesia

Anesthesia was done initially by premedication using 0.2 mg/kg atropine sulfate and 1.0 mg/kg diazepam, each was given intramuscular, continued by ketamine 20 mg/kg, and then maintained using additional 10 mg/kg dose if there was a reaction from the rabbits.

ASCs Preparation

The stem cells used were allogenic and were procured in advance of this study, Cryopreserved in the Airlangga University Stem Cell Research and Development Center, and thawed to be used for this study. It was retrieved from the scapular adipose tissue of New Zealand White Rabbits not included in this study, therefore the stem cells were allogenic. Lipoaspirates are collected in an aspiration container and may be comprised of three distinct layers: 1) an upper layer of oil due to the lysis of mature adipocytes, 2) a middle layer of adipose tissue, and 3) a bottom, liquid infranatant containing saline and contaminating cell types such as red blood cells (RBCs). Washing the lipoaspirate will remove the greater majority of contaminating red blood cells and saline. Following this initial culture period, the culture media may be aspirated, and any contaminating red blood cells gently removed by washing with sterile 1X PBS (Phosphate Buffer Saline). Replace with culture medium and continue to culture the ASCs as needed (Bhang et al., 2014; Jang et al., 2017).

The tissue cells were then processed and cultured until the 5^{th} passage. Phenotype confirmation was done using CD 45, CD 73, CD 90, CD 105 markers. We used 2 x 10^6 cells for each application in the stem cell group, and the cells were marked using PKH26 and traced using a fluorescence microscope.

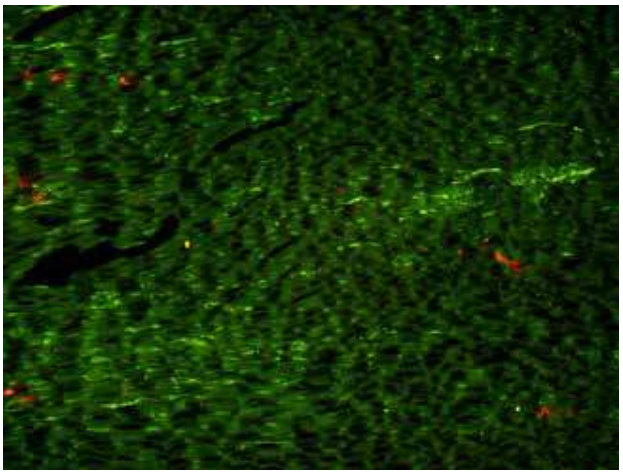

Figure 1. White arrow shows PKH26 stained cells (orange colored) within regenerated cartilage tissue

Secretome Preparation

Secretome used in this study were retrieved from the stem cells processing. Secretome was collected after 24 hours and centrifuged at 700 ×g for 8 min to remove cell debris. Retrieval was done in 70-80% stem cell confluent cultures. It was then centrifuged and used in a ±1 mm solution for the application in each treatment.

DFLP Scaffold Preparation

The base materials for this scaffold was taken from the femoral head and condyles of Ongole cattle aged 24 months. These cattle were certified healthy and retrieved from the Pegirian slaughterhouse in Surabaya. The cartilages were then separated from the subchondral bone by using bone rongeurs. The separated cartilage was flushed with NaCl 0.9% solution or distilled water until it was clean, and ground into powder. These powders were then mixed with NaCl 0.9% solution or distilled water under 1:1 ratio and then put into a 5 cm diameter mold.

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These molds were then deep-frozen in –80^{0}C for at least 24 hours. Afterward, it was dried by a sublimation technique using a freeze-dry machine. These dried molds were then re-molded into 5 mm diameters. The scaffold used in this study is as in Fig. 1. An earlier preliminary study has shown that this material was biocompatible and may be used as a biomaterial (Dwikora Novembri Utomo & Fachrizal, 2017; D. N. Utomo et al., 2019; Wirashada, Utomo, Purwati, Widhiyanto, & Hernugrahanto, 2019).

Figure 2. *DFLP Scaffold*

Articular Injury Model

The site used for the injury model is at the femoral trochlea. Surgery was done under standard sterile protocols. A midline incision and a medial parapatellar approach were done, and a full-thickness 4.5 mm^2 lesion was made. Each lesion was treated as per groups divided accordingly. In the control group, the defect was left as is. The surgeries were done by the author, experienced in the procedure. The timing of surgery was divided daily between groups, in which every group was done in a single day, followed by the other groups in respective days. We consider this to mimic microfracture by stimulating the subchondral bone marrow. When DFLP Scaffold was used, to fix the scaffold, a thin film of fibrin glue (Beriplast® P Combi-Set) was applied. These are shown in Fig. 2 The rabbits were then put inside cages and environment mentioned above until the 12th week. No specific immobilization system to the affected limb was applied to the animals. As the pain subsided, they start to mobilize with both legs inside their respective enclosures. They were then terminated on the 12th week each group according to the dates the surgery was done, and immunohistochemistry staining for collagen type I and type II was performed.

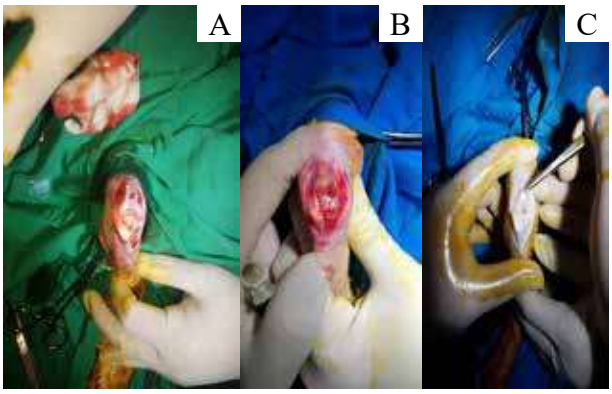

Figure 3. a. The defect on the femoral trochlea (Control group); b. Implanted scaffold (Scaffold group); c. Implanted scaffold saturated with ASCs/ Secretome (Scaffold + ASCs/Secretome group with similar clinical picture between both groups)

Statistical Analysis

We did a statistical analysis using SPSS 26. Data normality was then tested using Shapiro-Wilk. All collagen type I expression score has $p > 0.05$, thus considered having normal distribution, and further statistical analysis was done using Oneway ANOVA. Collagen type II expression for the K group has $p = 0.023$. The statistical analysis for the Collagen type II group was then done using Kruskall-Wallis.

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RESULTS

Table 1. IRS Scoring System, final score = A x B

A	B
0 pts – no cells with positive reaction 1 pt – to 10% cells with positive reaction 2 pts – 11-50% cells with positive reaction 3 pts – 51-80% cells with positive reaction 4 pts – > 80% cells with positive reaction	0 pts – no color reaction 1 pt – low intensity of color reaction 2 pts – moderate intensity of color reaction 3 pts – intense color reaction

Table 2. Collagen type I and type II mean comparison between groups

(K=Control, S=Scaffold, SA= Scaffold + ASCs, SS=Scaffold + Secretome)

	Group	Mean (points)	Std. Deviation	P-Value (α =0.05)
Col-1	K	3.33	2.04	0.546*
	S	4.30	2.00	
	SA	5.35	2.64	
	SS	3.85	2.05	
Col-2	K	2.20	1.17	**0,016#**
	S	4.17	2.73	
	SA	7.30	1.37	
	SS	6.35	2.35	

Note : *: *Oneway* ANOVA Test

#: Kruskal Wallis Test

Table 3. Kruskal-Wallis Pairwise Comparison Between Group for Collagen Type II

(K=Control, S=Scaffold, SA= Scaffold + ASCs, SS=Scaffold + Secretome)

Group Col-2	Statistic Test	Std. Error	Std. Statistic Test	Sig. (α =0.05)
K-S	-4.92	3.41	-1.44	0.149
K-SA	-11.25	3.81	-2.95	**0.003**
K-SS	-8.88	3.81	-2.33	**0.020**
S-SA	-6.33	3.81	-1.66	0.097
S-SS	-3.96	3.81	-1.04	0.299
SS-SA	2.38	4.18	0.57	0.569

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To evaluate the immunohistochemistry expression of collagen type I and type II, we use the (Immuno Reactive Score) IRS scoring system (Table 1). This is a semiquantitative scoring system in which the final score is a multiplication between the percentage of immunoreactive cells and the color intensity of immunoreactive cells. Each sample scores were the mean results of 5 different field of view in 1000x magnification.(Nowak, Madej, & Dziegiel, 2007; Remmele & Stegner, 1987). To avoid bias in evaluation, we do a blinded evaluation between two independent observers competent and experienced in the field of histology and cartilage.

Table 2 shows the mean values and standard deviation for each group's collagen type I and type II expression. The mean value for collagen type I for groups K, S, SA, and SS are as follows: 3.33±2.04, 4.50±2.00, 5.35±2.64, and 3.85±2.05. Collagen type I expression comparison between treatment groups shows no significant difference (p = 0.546). Collagen type II expression Kruskal-Wallis across groups comparisons shows the statistical difference between groups (p = 0.016), the analysis was then continued using Kruskal-Wallis pairwise comparison (Table 3) shows significant difference between K–SA (p = 0.003) and K – SS (p = 0.020).

The IHC scoring analysis correlates to the mean values of collagen type II groups. Scaffold + Stem Cell group and Scaffold + Secretome group shows better results than the control group. Fig. 3 shows normal cartilage, and Fig. 4 and 5 shows IHC evaluation for Collagen type I and Collagen type II for each group respectively.

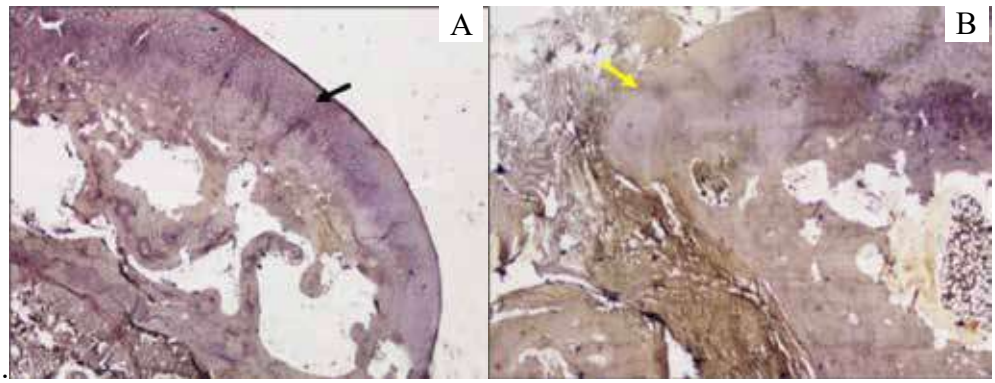

Figure 4. A. Normal Cartilage, B. Defect area. Black and yellow arrows were the areas of evaluation (Immunohistochemistry staining, 40x magnification; Nikon H600L microscope; DS Fi2 300 megapixel camera)

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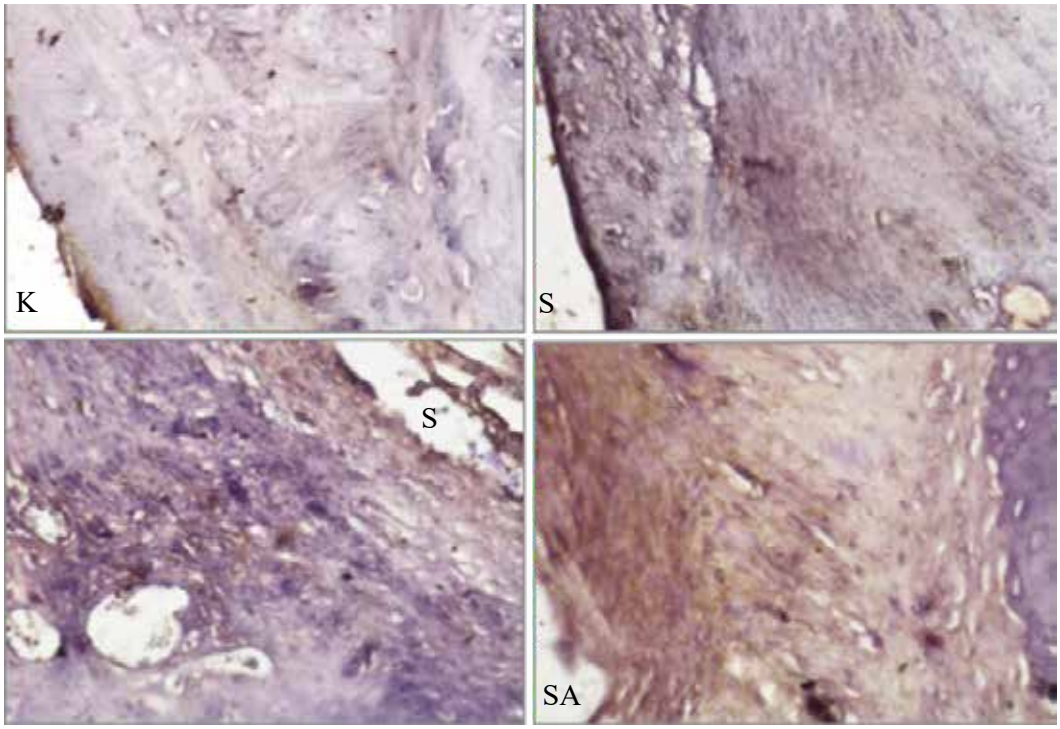

Figure 5. Comparison for Col-1 IHC staining for each group on chondrocytes and epiphyseal matrix for evaluation. (Immunohistochemistry staining, 40x magnification; Nikon H600L microscope; DS Fi2 300 megapixel camera)

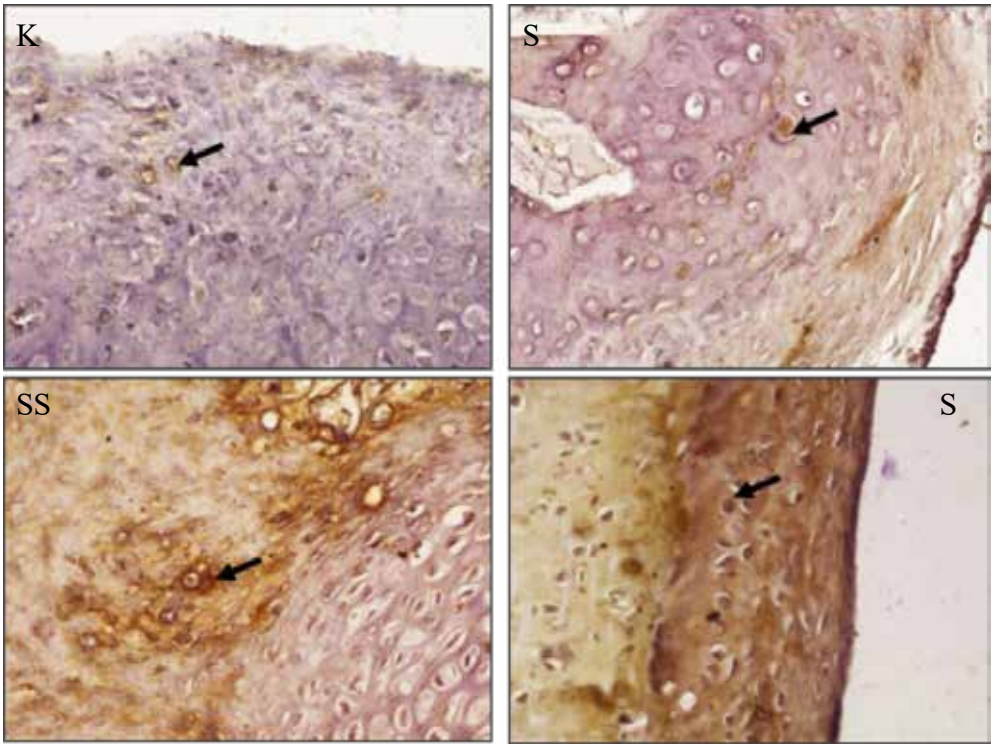

Figure 6. Comparison for Col-2 IHC staining for each group on chondrocytes and epiphyseal matrix for evaluation. (Immunohistochemistry staining, 40x magnification; Nikon H600L microscope; DS Fi2 300 megapixel camera)

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DISCUSSION

A Scaffold should be biodegradable, biocompatible, non-toxic, and has the ability to integrate with the host tissue and to sustain the life and phenotype of the cells during in-vitro and in-vivo implantation. Based on these results, the SA group has the highest mean value. For the collagen type II mean values for each group, K, S, SA, and SS are as follows: 2.20±1.17, 4.17±2.73, 7.30±1.37, and 6.35±2.35. These results showed that the SA group has the best mean value. DFLP Scaffold composite with ASCs or Secretome shows potential for cartilage regeneration therapy by increasing type II collagen expression as in hyaline-like cartilage.

Scaffold functions for cell isolation and to capture the extracellular matrix produced by the chondrocyte. Tissue engineering is considered to increase the mechanical characters of the end product. A chondro-inductive trigger is also needed to start chondrogenesis and chondrogenic characteristics of the implanted cells. DFLP Scaffold has been tested for biocompatibility, physicobiochemical properties, and its immunogenicity. Its results showed that DFLP Scaffold has the characteristics a scaffold should have. Compared with readily available synthetic scaffolds in the market, DFLP Scaffold is easier to produce and maybe a more economical alternative. In this study, we noted that there were no adverse effects related to the treatment (Dwikora Novembri Utomo & Fachrizal, 2017; D. N. Utomo et al., 2019; Wirashada et al., 2019).

Studies using secretome as biotherapy to overcome bone defects showed an increase of cartilage volume and quality, showed by mineralization and connectivity increase, and also angiogenesis. These results imply that the potential for secretome biotherapy for musculoskeletal problems, specifically, cartilage problems for this study (Khatab et al., 2018; Satue, Schuler, Ginner, & Erben, 2019).

The increase of cartilage formation might be caused by the decrease of chronic inflammation by secretome biotherapy. Secretome from various sources contains many growth factors that can promote migration, proliferation, and induction. After the discovery of mesenchymal stem cells (MSCs) in the bone marrow by Becker et al. in the 1960s, regenerative medicine discipline has developed. Many stem cells have been discovered since then. Adipose tissue has been proven to be a readily and abundant source for multipotent stem cells. These cell population of Adipose-Derived Stem Cells (ASCs) has also been proven to be safe (Satue et al., 2019; Vizoso, Eiro, Cid, Schneider, & Perez-Fernandez, 2017).

Adipose-derived stem cells (ASCs) were retrieved from adipose tissue that has the potency to differentiate into adipocyte and some other cells. ASCs are also similar to MSCs that can be cultured in-vivo or in-vitro for various therapy options, cartilage injuries included. ASCs were often taken from the white subcutaneous adipose tissue of the gluteus, abdomen, or infrapatellar fat pad by lipoaspiration or open excision. Subcutaneous adipose tissue contains adipocyte and a various stromal vascular fraction (SVF), including fibroblast, endothelial cells, pre-adipocyte, vascular muscle cells, monocyte, and ASCs themselves (Fukuda, Chikama, Nakamura, & Nishida, 1999; Khatab et al., 2018).

Cell-Based Therapies such as Autologous Chondrocyte Implantation (ACI) and its further developments and Bone Marrow Stimulation (BMS) such as Microfracture are types of surgical options for articular cartilage to achieve regenerated tissue with biomechanics and structure as close as normal hyaline cartilage. ACI and its developments

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give good results for cartilage lesions > 4 cm^2, whereas Microfractures are used for smaller lesions. Studies comparing these 2 techniques showed that long-term outcomes of these tissue engineering techniques do not show significant differences (Harrell et al., 2019; Jang et al., 2017).

The expected regenerated tissue after cartilage defect therapy is more hyaline-like cartilage and achieved with a single event surgery. This expectation gives rise to the usage of Scaffold + Stem cell/Secretome therapy for cartilage defects in this study. Other studies using scaffold primarily uses synthetic material; one of them uses atelocollagen and poly-L-lactic acid (PLLA), evaluated at the 12th week, hyaline-like cartilage was seen (Harrell et al., 2019; Xu et al., 2019).

In this study, we found that the secretome group also increases the proliferation of collagen type II compared to the control group. This may correlate with several growth factors identified in earlier studies within secretomes, such as PDGF-BB, TGFβ2, VEGF, TIMP-1, TIMP-2, angiogenin, and other unidentified factors such as exosomes. VEGF and angiogenin are potent inducers of angiogenesis, VEGF has also been proven to increase proliferation and osteoblast migration. PDGF-BB and TGFβ2 increase bone regeneration by acting as a chemotactic and proliferating agent for MSC recruited towards the defect to differentiate into osteoblast. Finally, TIMP-2 has also been proved to induce the proliferation of primary osteoblast culture in murine and increase bone defect healing. The in-vitro analysis showed that secretome therapy might induce cartilage regeneration (Kim, Kim, Lim, Lee, & Yun, 2010; Mancuso et al., 2019).

In this study, collagen type II expression is higher after scaffold + ASCs/secretome, and this has similar outcome as another study by Satue et al. (Satue et al., 2019) in which intraarticular MSCs injection was done to a murine knee full-thickness cartilage defect model, the study also found an increase of collagen type II expression compared to the control group (Buttgereit, Burmester, & Bijlsma, 2015; Janssens, ten Dijke, Janssens, & Van Hul, 2005). These results showed that stem cell also has the potential for regenerative therapy for chondrogenesis for cartilage defects. Neo-chondrogenesis is the expected result of multipotent MSC and Secretome therapy. In this study, we found that the addition of ASCs or Secretome in Scaffold therapy increases collagen type II expression significantly compared to the control group, although for collagen type I, there is no significant difference for its expression.

The limitation of this study is that we only evaluate two variables of determining the characteristics of regenerated cartilage tissue. Further studies regarding the orientation of the collagen in each layer of cartilage or other IHC markers should be done to further determine the characteristics of the regenerated cartilage tissue.

CONCLUSION

Implantation of DFLP Scaffold and supplemented with ASCs or Secretome in animal model shows a positive effect for cartilage defect treatment towards a hyaline-like cartilage result by increasing collagen type II expression.

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Research Article

Oxygen saturation among newborns in the first 10 hours of life to detect Critical Congenital Heart Disease - Ductus Dependent

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Abstract

Delay diagnosis of Critical Congenital Heart Disease (CHD) can be associated with sudden clinical deterioration and dangerous cardiovascular conditions. The oxygen saturation screening among newborns in the first 10 hours of life is essential for early detection of critical CHD. This study aims to prove that measuring oxygen saturation among newborns in the first 10 hours of life can detect critical CHD. This study is a diagnostic experimental with consecutive sampling subjects in the infant care unit of Dr. Soetomo Hospital, including all newborns with birth weight ≥ 1500 grams and oxygen saturation at ≥ 1 hour of age below 90%. The measurement of oxygen saturation uses fingertip pulse oximetry in the right hand and foot at the age of 10 hours. A "positive oxygen saturation" is defined as oxygen saturation ≤ 85% or different oxygen saturation ≥ 3%, while a "negative oxygen saturation" is when the oxygen saturation is 85% to 90% or different oxygen saturation is 3%. Echocardiography is performed for the gold standard. From November 2019 to January 2020, 11 newborns underwent an oxygen saturation examination. Five subjects (45.46%) in the category of positive oxygen saturation, echocardiographic showed all Critical CHD (100%). Six subjects (54.54%) with negative oxygen saturation category, echocardiographic results showed two critical CHD (33.34%) and four non-critical CHD (66.66%). Fisher's exact test $p < 0.005$ (α). The diagnostic oxygen saturation test among newborns at 10 hours of life shows ≤85%, all subject's echocardiography (100%) shows detection of critical CHD, while saturation 85% to 90% has of 33.3% for detection of critical CHD. The sensitivity and specificity of oxygen saturation for early diagnosis of critical CHD are 100% and 67%, respectively.

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INTRODUCTION

Routine neonatal inspection fails to detect more than 50% of infants with congenital heart disease (CHD). More than 55% of neonates with CHD do not show symptoms of murmurs in the nursery, and about 82% are discharged before diagnosis results are obtained, so this will increase mortality and morbidity (Du et al., 2017). More than 50% of infants with Critical CHD die at home or emergency room before the diagnosis is established, and every year 100-200 infants die in America due to the unknown critical CHD (Goetz, Elizabethm; Hokanson, 2012). Based on Riskesdas 2007 data, the most common cause of infant death in neonates 0 - 6 days is due to cardiovascular disorders (35, 9%) (Chalid, 2014).

Pulse oximetry screening in newborns has been shown to increase the detection of Critical CHD (Narvey et al., 2017). However, the official pulse oximetry screening protocols for CHD by the SACHDNC (Secretary's Advisory Committee on Heritable Disorders in Newborns and Children), AAP (American Academy of Pediatrics), AHA (American Heart Association), and the CDC (Centers for Disease Control and Prevention) recommend pulse oximetry measurements before discharge from the hospital limited to ages 24 to 48 hours (Goetz, Elizabethm; Hokanson, 2012; Engel & Kochilas, 2016). While other screening has inconsistent variations in pulse oximetry testing time, giving rise to variations in the diagnostic accuracy of pulse oximetry screening (Du et al., 2017). On the other hand, critical CHD requires early detection and surgical or non-surgical intervention in the first year of life to sustain life (Du et al., 2017), because its systemic or pulmonary circulation which cannot tolerate the transition from fetal circulation to serial circulation after birth and will depend on central shunts, especially patent ductus arteriosus (PDA) (Lee, 2010).

Delay in the diagnosis of critical CHD can be associated with sudden clinical deterioration, dangerous cardiovascular conditions, collapse, heart failure, end-organ damage, and even death (Movahedian et al., 2016; Du et al., 2017). Infants with duct-dependent lesions will experience severe desaturation, shock, or collapse when the PDA closes within hours or days after birth due to systemic hypoperfusion. These lesions include ductal pulmonary and systemic circulation (Lee, 2010; Tomar, 2016).

Congenital heart disease is reported to occur around 6 to 8 per 1000 live births. Critical CHD occurs in 2.5 to 3 per 1,000 live births (Du et al., 2017). About 15% of all CHDs are cyanotic, and about 30% of these cyanotic lesions are critical and potentially fatal lesions without treatment (Tsuda, 2016). However, until no,w there has never been a study of a critical CHD screening program with pulse oximetry in the first 10 hours of life. It is necessary to measure oxygen saturation with pulse oximetry based on the initial time of functional closure of the ductus arteriosus lumen (the first 10 hours of life) to get an accurate, early, and effective screening program; and assess its sensitivity and specificity to the gold standard of critical CHD by echocardiography. This study aims to prove that measuring oxygen saturation among newborns in the first 10 hours of life can detect critical CHD. The benefit is to reduce mortality and morbidity of critical CHD in neonates. Our research is in line with the 2016-2030 SDGs target: reduce infant and toddler mortality, by reducing neonatal mortality by 12 per 1,000 live births; reduce 1/3 of premature deaths due to non-communicable diseases (Ermelana, 2017).

METHODS

This study is a diagnostic test – experimental for newborns treated by selecting consecutive sampling subjects in the Neonatal Care Unit of Dr. Soetomo Hospital; which included, oxygen saturation of infants aged over 1 hour < 90%, birth weight ≥1500 grams, and parents of

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infants agreed to join the study. Infants who did not qualify the study criteria were excluded in the study.

Researchers made informed consent, observed, and recorded oxygen saturation with fingertip pulse oximetry Onyx II® (Medical USA) attached to the right palm or foot. The recording of maximal oxygen saturation values includes pre-ductal and post-ductal with stable pulse frequencies when subjects are calm, and monitoring lasts for about 1 to 2 minutes.

A positive result is defined as the oxygen saturation in the first 10 hours of life in the hands or feet is ≤ 85% or the difference in oxygen saturation between the right hand and foot ≥ 3%. Whereas, the negative result is when the oxygen saturation in the right hand or foot is > 85 - < 90%, or the difference in oxygen saturation between the right hand and the foot < 3%. Echocardiography as the gold standard is performed after the results of oxygen saturation with fingertip pulse oximetry is obtained.

Approval of research ethics was obtained from the Health Research Ethics Commission Dr. Soetomo Surabaya. Certificate of Passing Ethics Review Number 1621/KEPK/XI/2019, dated 1 November 2019.

Data processing was performed with the SPSS computer program version 17.0. Assessment of diagnostic tests by tabulating negative and positive detection results in the 2x2 table was used for the Fisher extract test. The determined sample size (n), p values, α, and critical values are presented in tables. The results are the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). A value of $p < 0.05$ was considered statistically significant. The data is then presented descriptively in tabular and narrative form. The sample size in this study uses the formula n = factor level 1 x factor level 2 x constants; n ≥ 10 (Sarmanu, 2017).

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RESULTS

Table 1. Subject Characteristics

Characteristics	Amount (n=11)	Presentation
Sex		
Boys	7	64%
Girls	4	36%
Gestational Age		
< 37 weeks	3	27%
37-42 weeks	8	73%
>42 weeks	0	0%
Delivery		
Spontaneous	6	55%
Caesarian	5	45%
Forceps or Vacuum	0	0%
Weight born (gram)		
< 2500	5	45%
2500-4000	6	55%
> 4000	0	0%
Apgar score first 1 minutes		
< 6	2	29%
≥ 7	9	71%
Family history in CHD		
Yes	0	0%
No	11	100%
Diagnosis of antenatal CHD		
Yes	0	0%
No	11	100%
Other congenital abnormality		
Yes	1	10%
No	10	90%
Murmur		
Yes	4	36%
No	7	64%

Table 2. Oxygen saturation of pulse oximetry and echocardiography result

No	S	Sex	Cyanosis	Murmur	Sat pre	Sat post	Δ Sat	SpO2	Echo
1	SI	L	-	+	87	85	2	-	-
2	LAF	L	-	+	71	72	1	+	+
3	FJP	P	-	+	86	84	2	-	+
4	EN	L	+	-	73	73	0	+	+
5	FA	L	-	+	73	74	1	+	+
6	SS	L	-	-	89	89	0	-	+
7	EPW	P	-	-	89	89	0	-	-
8	NO	L	+	-	79	78	1	+	+
9	DR	L	+	-	76	75	1	+	+
10	DN	P	-	-	88	88	0	-	-
11	NWM	P	-	-	88	88	0	-	-

S: Subject, Sat pre: pre-ductal saturation, Sat post: post-ductal saturation, Δ Sat: difference between pre-ductal and post-ductal saturation, SpO2: oxygen saturation, Echo: echocardiography, +: CCHD, -: not CCHD

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Table 3. Compatibility of Echocardiography with Oxygen Saturation

Result of Echocardiography	Oxygen Saturation		Total
	5 infants (+)	6 infants (–)	
+	5 (a)	2 (b)	7 (a + b)
-	0 (c)	4 (d)	4 (c + d)
Total	5 (a + c)	6 (b + d)	11 (n)

The type of CHD that can be indicated by oxygen saturation

In this study, there were 11 newborns examined using fingertip pulse oximetry. The positive detection results obtained in five subjects (46%), and the echocardiographic confirmed all (100%) have critical CHD. While the negative detection results were obtained in 6 subjects (54%), and the echocardiography showed 33.33% confirmed critical CHD and 66.67% non-critical CHD. The data are shown in Table 2.

Diagnostic Value of Oxygen Saturation - Fingertip Pulse Oximeter

The results of detecting oxygen saturation from fingertip pulse oximetry on echocardiography in diagnosing congenital heart disease depend on the duct by using the Fisher test and the 2x2 table are shown in Table 3.

From Table 3, can be calculated:

- Sensitivity : a/(a+c) = 1
- Specificity : d/(b+d) = 0.67
- Positive predictive value (PPV) : a/(a+b) = 0.71
- Negative predictive value (NPV) : d/(c+d) = 1
- Positive likelihood ratio (PLR) : a/(a+c) : b/(b+d) = 3.33
- Negative likelihood ratio (NLR) : c/(a+c) : d/(b+d) = 0

Limitations of Oxygen Saturation-Fingertip Pulse Oximetry Test in Infants

Examination of oxygen saturation with a fingertip pulse oximeter (Onyx II ®) takes longer to achieve maximum results, and the baby must be in a calm condition.

DISCUSSION

Advantages and Limitations of this study

This research is the first attempt to apply oxygen saturation screening with pulse oximetry for the early detection of critical CHD among newborns at the first 10 hours of life at a hospital in Indonesia. Many hospitals in developed countries have routinely implemented oximetry screening to prevent neonatal morbidity and mortality due to late critical CHD diagnosis. In Indonesia, this has not yet become an official policy of hospitals and related government institutions. Critical CHD screening using pulse oximetry according to AAP recommendations requires expensive equipment (Miller et al., 2016); therefore the use of fingertip pulse oximeter (which is more affordable) is expected to be a breakthrough effort so that screening can be widely applied in limited facilities both hospitals and other health care centers in Indonesia.

In addition to the above advantages, the oxygen saturation with fingertip pulse oximeter has limitations in terms of tool specifications. This type of oximeter can work well on fingers with a thickness of 8-26 mm, so it is more appropriate for use in pediatric-adults. However, a research shows that the use of fingertip pulse oximeter in newborns has good results (Phattraprayoon et al., 2012).

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Characteristics of Subjects

The age of the subjects in this study was 10 hours. The age limit was chosen because it is related to the time of constriction and closure of the ductus arteriosus in term infants. It is well known that the initial 'functional' closure of the ductus arteriosus lumen is in the first 10-18 hours of life, so that perhaps at that time, there has been a hemodynamic change that causes desaturase. Functional closure of the ductus arteriosus is up to 72 hours after birth, followed by the anatomical closure process at the age of 3-4 weeks after birth. In premature neonates, the mechanism of the closure of the ductus arteriosus occurs more slowly, within two days in most premature infants even up to the age of 4-12 months (Clyman, 2006; Gournay, 2011; Ontoseno, 2014; and Chacko et al., 2016). In addition, a premature examination can increase false-positive results due to the circulation of the fetal to neonatal transition and stabilization of systemic oxygen saturation levels. If the examination is carried out later, then the opportunity for CHD intervention before the duct closes can be missed (Mahle et al., 2009; Kemper et al., 2011). A systematic review showed that examinations at the age of >24 hours decreased the false positive rate from 0,87% to 0.035% (Mahle et al., 2009). Another study found lower false positives at> 24 hours of age than <24 hours of age (0.05% [95% CI 0.02-0.12%] compared to 0.5% [95% CI 0.29- 0,86%]; p = 0,0017) (Thangaratinam et al., 2012).

The median gestational age in this study was 38 weeks (37 weeks minimum and 41 weeks maximum. Another study involved infants with gestational age ≥37 weeks (Riede et al., 2010), and ages >34 weeks and ≥35 weeks (Ewer et al., 2011; Bradshaw et al., 2012). Gestational age is related to the time of constriction and closure of the ductus arteriosus. In full-term infants, functional ductal closure occurs within 24 hours, whereas in preterm infants, the duct is more likely to remain open because the ductal smooth muscle has no constrictor response to fully developing oxygen (Park, 2008).

In this study, prior to pulse oximetry examination, no subjects diagnosed with CHD during antenatal examination with ultrasound and murmurs were shown in 4 subjects out of 11 subjects who showed CHD. The study of de-Wall Granelli et al. by including a much greater number of subjects received a low incidence of hearing cardiac noise that was 9/38374 subjects (Granelli et al., 2009). Riede et al. also got a low incidence of subjects who showed symptoms of cardiovascular abnormalities before the pulse oximetry examination that was 18/48384, while subjects who received a diagnosis of antenatal CHD were 54/48384 (Riede et al., 2010).

Incidence Rate and Type of Critical CHD.

Pulse oximetry screening in newborns aims to detect critical CHD before the onset of clinical manifestations before the baby is discharged from the hospital, thereby reducing neonatal morbidity and mortality. Various ductal-dependent abnormalities are expected to be detected, such as left heart hypoplasia syndrome, pulmonary atresia (with intact septum) total anomalous pulmonary venous drainage, transposition of large arteries, tricuspid atresia, or truncus arteriosus (Madjus & Abou Al-Seoud, 2014).

In this study, oxygen saturation screening with pulse oximetry can find subjects with critical CHD, which is likely influenced by the lowering of the oxygen saturation target. In the Sendelbach et al. study, there were no subjects diagnosed with critical CHD through pulse oximetry screening (Sendelbach et al., 2008). The study of Koppel et al. showed a prevalence of critical CHD 1 per 564 births, and in an asymptomatic population undergoing screening

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1 per 2256, while the number of critical CHD that was successfully detected with oximetry was 3/11,281 or 1 per 3760. The study found 2 cases of total anomalous venous drainage pulmonary and 1 case of truncus arteriosus (Koppel et al., 2003). Ewer et al. obtained a prevalence of 2.6 per 1000 life disorders (53/20,055) cases of major heart abnormalities (Ewer, Andrew K; Middleton, Lee J; Furmston, Alexander T; Byohar, Abhay; Daniels, Jane P; Thangaratinam, 2011).

Oxygen Saturations Pulse Oximetry Diagnostic Value

In this study, the median pre-ductal oxygen saturation in the right hand was 86% and post-ductal in the leg 84% in 10-hour-old infants, with an average oxygen saturation of 85.4%. The Poets et al. study found results with a median oxygen saturation of 97.6% in infants aged 2-7 days (Poets CF, Stebbens VA, Lang JA, O'Brien LM, Boon AW, 1996). Lavesque et al. received a median oxygen saturation of 97% with a mean of 97.2% in all newborns in rooming-in/bedding-in (Levesque et al., 2000).

In this study, none of the subjects differed between pre- and post-ductal > 3%. Ewer et al. obtained 195/20,055 (0.97%) of newborns with abnormal oxygen saturation for CHD by pulse oximetry examination (Ewer et al., 2011). Sendelbach et al. received results of 15,233 subjects who underwent an oximetry examination; only one subject showed positive detection results and normal echocardiographic examination results. No subject with critical CHD was detected through oximetry screening. The study included a large number of subjects, sensors attached to the feet, screening time when the subject was 4 hours old, abnormal oxygen saturation limits <96%, screening was repeated when the subject was about to leave the hospital (Sendelbach et al., 2008).

In this study, the results of the oximetry test with fingertip pulse oximetry (Onyx II ®) at the age of 10 hours of life were compared with the gold standard that is echocardiography to detect critical CHD. In this study, at 10 hours of age, oxygen saturated subjects at extremities < 85% echocardiographic results 100% showed Critical CHD, while subjects with oxygen saturation ≥85% to <90%, echocardiographic results 33.33% showed Critical CHD and 66.67% showed CHD that did not critical. The sensitivity of oxygen saturation in diagnosing critical CHD in newborns at 10 hours is 1. The specificity of oxygen saturation in diagnosing Critical CHD in newborns at ten hours of life is 0.67. The positive predictive value of oxygen saturation in diagnosing Critical CHD in newborns at 10 hours of age is 0.71. The negative predictive value of oxygen saturation in diagnosing Critical CHD in newborns at 10 hours of age is 1. The ratio of the positive likelihood of oxygen saturation in diagnosing Critical CHD in newborns born at the age of 10 hours is worth 3.33. The negative likelihood ratio of oxygen saturation in diagnosing Critical CHD in newborns at 10 hours is 0. Sensitivity results that vary, which cannot be assessed up to 100%, are shown by a systematic review of Mahle et al. The difference in methodology seems to affect these results. In addition, the review also received a high specificity of 95.5% to 100% (Mahle et al., 2009). Another systematic review by Thangaratinam et al. in 13 studies found moderate sensitivity (76.5% (95% CI 67.7-83.5%)) with high specificity (99.9% (95% IK 99, 7 -99.9%)) (Thangaratinam et al., 2012).

Limitations of Oxygen Saturation Fingertip Pulse Examination in Newborns

Oxygen saturation with pulse oximetry is performed when the baby is calm; there is no excessive movement, fighting, or crying. This study found that fingertip pulse oximetry

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examination was needed to achieve maximum results. Reading the results of oxygen saturation measurements on the monitor screen can reach a constant value ,usually takes 10-30 seconds (Pullen, 2010). A research `using Masimo SET® in newborns shows the average total time needed to achieve accurate data is 25 (± 7) seconds (O'Donnell et al., 2005). Some factors that can affect the length of time the results are achieved are the tool, baby, and examiner (Bradshaw et al., 2012). Researchers encountered obstacles when carrying out oxygen saturation, which resulted in repeated oxygen saturation in order to achieve maximum results. These obstacles are related to the tool and baby factors. Fingertip pulse oximeter is a type of conventional oximeter with technology that is not designed to be resistant to movement.

CONCLUSION

In this study, oxygen saturation screening with fingertip pulse oximetry in newborns at 10 hours of age shows that oxygen saturation ≤ 85% all showed critical CHD and on oxygen saturation >85% to <90% showed 33.33% critical CHD, and 66.67% are not critical CHD. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, pretest probability, and post-test probability of pulse oximetry fingertip (Onyx II ®) compared to echocardiography for early detection of critical CHD in infants newborn has significant value. The measurement of oxygen saturation with fingertip pulse oximetry in newborns at 10 hours of age with the oxygen saturation ≤ 85% reflects the presence of critical CHD in newborns. Fingertip pulse oximetry examination in newborns has limitations mainly related to the influence of the baby's movements and tool factors.

Fingertip pulse oximeter can be an alternative of oximetry device for critical CHD-duct dependent screening in the newborns; however, it is worth noting the limitations of the tool above. Research with a greater number of subjects by screening pulse oximetry in all newborns at the age of 10 hours in a hospital in a rooming-in/bedding-in.

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Research Article

Effects of prebiotics, probiotics, and synbiotics on the body weight, blood glucose, triglyceride and TNF-α of diet-induced obesity rats

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Abstract

High-fat diet leads to obesity-associated chronic low-grade inflammation. Prebiotics, probiotics, and synbiotics produced short-chain fatty acids (SCFA), bonded to *G protein-coupled receptors* (GPR)-41 and GPR-43 decreased triglyceride deposits in adipocytes and liver, decreased fatty acid oxidation, increased glucose regulation and insulin sensitivity thus reduced the risk of obesity and metabolic syndrome. This study conducted in order to evaluate the effects of prebiotics, probiotics, and synbiotics on the body weight, blood glucose, triglyceride, and TNF-α used rats model, which were fed by a high-fat diet. Thirty-eight 6-8 weeks old male rats were fed by high-fat diet for three weeks, then rats were randomly divided into four groups, high-fat diet (HFD), a high fat diet with prebiotics supplementation (HFD+ PRE), a high fat diet with probiotics supplementation (HFD+PRO), and high-fat diet with synbiotics supplementation (HFD+SYN) for three weeks. Blood samples and body weight were measured at the third and sixth week. There was no effect of prebiotics, probiotics, and synbiotics on body weight, triglyceride levels, blood glucose, and TNF-α in rats fed a high-fat diet compared to control. These results suggested that supplementations gave inconsistent results with other studies and needed further researches.

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INTRODUCTION

The prevalence of obesity in adulthood had doubled since 1980, estimated in 2015, one third of the world's population suffered from obesity (Chooi, Ding, & Magkos, 2018). Consumption of high fat diet was one of the causes of obesity (Xu & Xue, 2016).

Obesity caused inflammation of adipose tissue called meta-inflammation (Reilly & Saltiel, 2017), released pro-inflammatory cytokines interleukin (IL)-12, IL-17, IL-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ thus promoted M-1 polarization (Li et al., 2018), so TNF-α was one of the cytokines closely related to metabolic syndrome (Indulekha, Surendar, & Mohan, 2011).

TNF-α, IL-6, leptin, and free fatty acids (FFA) caused insulin receptor substrate (IRS)-1 and (IRS)-2 degradation, promoted insulin resistance (Kwon & Pessin, 2013) characterized by the decrease of glycogenesis and glucose uptake, and lipolysis (Samuel & Shulman, 2016).

The effects of prebiotics, probiotics, and synbiotics in obesity are increasingly being studied. Supplementations produced short-chain fatty acids (SCFA), bonded to *G protein-coupled receptors* (GPR)-41 and GPR-43 decreased triglyceride deposits in adipocytes and liver, decreased fatty acid oxidation, increased glucose regulation and insulin sensitivity (Winer, Luck, Tsai, & Winer, 2016) (Tunapong et al., 2018) (Markowiak & Ślizewska, 2017).

However, other studies mentioned opposite results (Luo, Yperselle, Rizkalla, Rossi, & Bornet, 2000) (Million et al., 2012), so that supplementations required further researches before established it as an additional therapy for obesity.

METHODS

Animals

Adult Male Wistar rats (Marques et al., 2016) (n = 18), 6-8 weeks, were obtained from the Biochemistry Department (Universitas Airlangga, Indonesia). All rats were caged with a 12 h light/dark cycle, fed by standard diet, and water *ad libitum*. After a week of adaptation, all rats were fed by a high-fat diet for three weeks. Then, rats were randomly divided into four groups, high-fat diet (HFD); high-fat diet + prebiotics supplementation (HFD+ PRE); high-fat diet + probiotics supplementation (HFD+PRO); and high-fat diet + synbiotics supplementation (HFD+SYN) for three weeks. Bodyweight, triglyceride, blood glucose, and TNF-α were measured at the end of the third and sixth week when the highest effects occurred. Ethical approval was obtained from the Health Research Ethical Clearance Commission, Universitas Airlangga Faculty of Dental Medicine No. 534/HRECC.FODM/VII/2019.

Standard diet

Standard diet used comfeed 593® (PT. Charoen Pokphand Indonesia). Feed compositions were protein (15%), fat (3%), fiber (8%), and ash (6%). Every rat was given a standard diet everyday *ad libitum* intended to gain the rat's nutritional needs that couldn't be obtained by providing a high-fat diet.

High-fat diet

Cow brain 1 g/rat/day (Abdel-Hafez, Othman, & Seleim, 2011) and egg yolk powder 0.5 g/rat/day diluted with aquadest. Rats were fed by using gastric tube 2 ml/rat/day (Alioes, Sukma, & Sekar, 2019). High-fat diet administration was given every day at 9.00 am.

Supplementations

Prebiotics consisted of mix FOS and GOS (0.5 g/kg body weight/day) (Kao, Spitzer, Anthony, & Lennox, 2018). Probiotics consisted of 1 x 10^{10} CFU/ml mix strain *Lactobacillus casei,*

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Lactobacillus rhamnosus, Lactobacillus acidophilus, and *Bifidobacterium spp.* (10 ml/ kg body weight/day) (Nimgampalle & Kuna, 2017). Both were obtained from the Faculty of Sains and Technology (Universitas Airlangga, Indonesia). Synbiotics were made by mixing both ingredients. Supplementation was administered by using a gastric tube right after high-fat diet administration to reduce stress.

Bodyweight measurement

The rat was put on the plastic bowl on the weighing scales, and carefully adjusted the weight.

Blood collection

Two cc blood was collected in a red-topped tube, put in the cool box, and transferred to the laboratory. Blood samples were centrifuged at 2,000 x g, 4º C for 10 minutes to obtain blood serum.

Serum analysis

Triglyceride and blood glucose were performed in a spectrophotometer using Rajawali commercial kit no. 116392® GPO PAP method and no. 112191® GOD PAP method. TNF-α was measured in the ELISA-Sandwich method used Elabscience® reagen.

Data analysis

Pre and post-test data were analyzed using a *paired t-test* (if data were normally distributed) or *Wilcoxon test* (if data weren't normally distributed). Comparative tests between the control group and supplementation groups used *independent t-test* (if data were normally distributed) or *Mann Whitney* (if data weren't normally distributed). Statistical tests used SPSS version 22, p-value < 0,05 was considered as a significant value.

RESULTS

Supplementations effect on body weight

A high-fat diet increased body weight before and after the intervention. The supplementation of prebiotics, probiotics, and synbiotics could not control the increase of body weight caused by a high-fat diet ($p < 0,01$) so that until the third week of treatment, there was still an increase in body weight (Table 1). There were no significant differences in body weight between the control and treatment groups (Table 2)

Supplementations effect on blood glucose

Supplementation of prebiotics, probiotics, and synbiotics did not change glucose levels. There was only a downward trend of glucose level in HFD+PRE and HFD+SIN groups (Table 3). There were no significant differences in blood glucose levels between the control and treatment groups (Table 4).

Table 1. Effect of supplementations on body weight within groups

Groups	Pre-supplementations	Post-supplementations	p-value
	Mean ± SD	Mean ± SD	
HFD	174.57 ± 29.478	235.29 ± 41.299	0,011
HFD + PRE	176.71 ± 24.336	224.71 ± 26.329	0,001
HFD + PRO	159.44 ± 25.870	213.89 ± 51.910	0,001
HFD + SIN	190.78 ± 31.352	253.44 ± 48.161	0,001

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Table 2. Effect of supplementations on body weight among groups

Groups	Δ Body Weight	p-value
	Mean ± SD	
HFD	60,71 ± 44,195	0,337
HFD + PRE	48,00 ± 19,079	
HFD	60,71 ± 44,195	0,461
HFD + PRO	54,44 ± 30,373	
HFD	60,71 ± 44,195	0,628
HFD + SIN	62,67 ± 38,380	

Table 3. Effect of supplementations on blood glucose within groups

Groups	Pre-supplementations	Post-supplementations	p-value
	Mean ± SD	Mean ± SD	
HFD	176.29 ± 29.607	147.86 ± 15.453	0,118
HFD + PRE	179.86 ± 47.386	135.43 ± 22.315	0,0502
HFD + PRO	168.44 ± 28.426	172.67 ± 21.413	0,105
HFD + SIN	168.56 ± 33.201	144.56 ± 11.865	0,755

Table 4. Effect of supplementations on blood glucose among groups

Groups	Δ Blood glucose	p-value
	Mean ± SD	
HFD	-28,43 ± 41,299	0,949
HFD + PRE	-44,43 ± 48,100	
HFD	-28,43 ± 41,299	0,550
HFD + PRO	4,22 ± 39,280	
HFD	-28,43 ± 41,299	0,668
HFD + SIN	-24,00 ± 39,446	

Table 5. Effect of supplementations on triglyceride within groups

Groups	Pre-supplementation	Post-supplementation	p-value
	Mean ± SD	Mean ± SD	
HFD	17,365 ± 3,077	21,285 ± 6,111	0,271
HFD + PRE	17,259 ± 5,646	17,003 ± 3,466	0,499
HFD + PRO	17,347 ± 6,882	20,546 ± 8,318	0,400
HFD + SIN	17,251 ± 1,450	30,568 ± 23,600	0,058

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Table 6. Effect of supplementations on triglyceride among groups

Groups	Δ Trigliserida *Mean* ± SD	p-value
HFD	9.29 ± 23.556	0,848
HFD + PRE	-20.00 ± 90.618	
HFD	9.29 ± 23.556	0,169
HFD + PRO	13.33 ± 34.681	
HFD	9.29 ± 23.556	
HFD + SIN	-19.22 ± 25.044	0,900

Table 7. Effect of supplementations on TNF-α within groups

Groups	Pre-supplementation *Mean* ± SD	Post-supplementation *Mean* ± SD	p
HFD	17,3649 ± 3,07651	21,2849 ± 6,11110	0,176
HFD + PRE	17,2589 ± 5,64610	17,0030 ± 3,46623	0,866
HFD + PRO	17,4369 ± 6,88219	20,5463 ± 8,31814	0,314
HFD + SIN	17,2514 ± 1,44980	30,5678 ± 23,60047	0,008

Table 8. Effect of supplementations on TNF-α among groups

Groups	Δ TNF-α *Mean* ± SD	p
HFD	3,9200 ± 7,32187	0,915
HFD + PRE	-2,559 ± 6,59710	
HFD	3,9200 ± 7,32187	0,742
HFD + PRO	3,1094 ± 7,79678	
HFD	3,9200 ± 7,32187	0,266
HFD + SIN	13,3163 ± 23,73930	

Supplementations effect on triglyceride

High-fat diet administration for 3 weeks had not been able to give an effect of increasing triglyceride levels. Most are still in the normal range of 82.70 ± 7.60 (Mesomya, Hengsawadi, & Cuptapun, 2001) (Ihedioha, Noel-uneke, & Ihedioha, 2013) (Table 5), so there was no difference between the control and treatment groups in reducing triglyceride levels (Table 6).

Supplementations effect on TNF-α

Gastric sonde to administer a high-fat diet caused an increase in TNF-α levels. This was also seen in the control group. The supplementation of prebiotic, probiotic, and synbiotic had not been able to reduce TNF-α levels due to gastric sonde installation; even there was a significant increase in the HFD + SIN group (p = .008) (Table 7). No significant differences were found between the control and treatment groups in TNF-α levels (Table 8).

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DISCUSSION

Effects of Probiotics, Prebiotics, and Synbiotics on Body Weight

Probiotics had been widely used for the treatment of diarrhea and inflammatory bowel disease, but some researchers revealed other uses of probiotics on weight loss (Karimi et al., 2015) (Paturi et al., 2015) (Nicolucci et al., 2017). Debates continued to emerge regarding the anti-obesity effect; another meta-analysis study actually stated the opposite, said that probiotics actually caused weight gain (Million et al., 2012). The weight gain effect might be influenced by the type of bacterial strain.

This study used a combination of several different species and genera of bacteria because it was more effective than a single strain (Chapman, Gibson, & Rowland, 2011), but other studies revealed that *Lactobacillus acidophilus* actually caused weight gain (Arora et al., 2012) (Million et al., 2012), whereas *Bifidobacterium spp* has the opposite effect (Ji et al., 2019), so there was no anti-obesity effect.

Another lack of the trial was a relatively short time of high-fat diet administration. An increase in body weight was estimated to continue within a period of 0-6 months. The first two months were the fastest, the next two months slowed down, and the lowest effect occurred in the last two months (Hafizur, Raza, Chishti, Shaukat, & Ahmed, 2015). In this study, rats were given a high-fat diet only three weeks, so the treatment did not provide the expected results because the weight gain effect still occurred. After 3 weeks of administration, weight was within the normal range (Nistiar, Racz, & Novakova, 2012) and likely to increase until six months ahead.

Supplementation of prebiotics, probiotics, and synbiotics reduced body weight due to increase secretion of anorexic hormones GLP-1 and PYY due to SCFA binding to their receptors, which decreased appetite (Tolhurst et al., 2012) (Fukui et al., 2018). The gastric tube was used to administer a high-fat diet, so there is no effect of decreasing appetite because the number of diets given every day was constant.

Effects of Probiotics, Prebiotics, and Synbiotics on Triglycerides

Supplementations reduced triglyceride levels (Choi et al., 2016) (Miao et al., 2016) through AMPK phosphorylation (den Besten et al., 2013), which triggers lipid oxidation (Jeon, 2016). However, based on existing reference values, rat triglyceride levels were within the normal range of 82.70 ± 7.60 (Mesomya et al., 2001) (Ihedioha et al., 2013). 3 weeks administration period was not enough to induce hypertrygliceride, which began to increase at week 9 (Marques et al., 2016).

Effects of Probiotics, Prebiotics, and Synbiotics on Blood Glucose

Decreased blood glucose levels caused by SCFA, caused AMPK phosphorylation leading to GLUT-4 translocation to the plasma membrane, which increased glucose uptake to cells (Jeon, 2016).

Glucose and triglyceride levels only experienced a downward trend, because rats were not yet in hypertriglyceridemia and hyperglycemic condition, so supplementation had only a slight effect when compared to the opposite situation, according to the other study stated that the magnitude of the effect was determined by hyperglycemic conditions before the intervention (Ruan et al., 2015).

Effect of Probiotics, Prebiotics, and Synbiotics on TNF-α levels

Gastric sonde used to administer high-fat diets or supplementations of prebiotics, probiotics, and synbiotics caused an increase in cortisol,

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and TNF-α levels indicated a response to stress (Lalive et al., 2002) (Walker et al., 2012). There was an upward trend in almost all groups. The increase of TNF-α was not a marker of adipocyte tissue inflammation but only a response to stress due to gastric sonde installation. In addition, the installation of gastric sonde could also cause death due to aspiration into the respiratory tract. A total of 4 rats died as a result of gastric distension, which triggered aspiration into the lungs (Damsch et al., 2011).

Use of TNF-α for monitoring the therapeutic effect of prebiotic, probiotic, and synbiotic was inappropriate because TNF-α levels do not accurately correlate with changes in fat mass (Bedoui et al., 2005) (Wu et al., 2016), lipid levels (Reinehr et al., 2005), and blood glucose (Choi et al., 2004). While other pro-inflammatory cytokines CRP, are more significantly correlated with BMI, blood pressure (Koenig et al., 1999), triglycerides (Yudkin et al., 1999), glucose (Bahceci et al., 2005), and obesity (Marques -vidal et al., 2012) (Fernandez-Berges et al., 2014).

CONCLUSION

Previous studies suggested that supplementations gave inconsistent results and needed further researches to establish a standard regarding dosage, time of administration, bacterial strains, and type of prebiotics before took prebiotics, probiotics, and synbiotics as an alternative therapy for the obesity problem. A high-fat diet and supplementation might have been too short of making any significant effect. Diet administration through oral (*ad libitum*) or gastric sonde should take into consideration, related to outcomes that influenced the results and implementation to humans.

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Research Article

Medical students' perspectives about distance learning during the early period of COVID 19 pandemic: A qualitative study

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Abstract

Medical education throughout the world has experienced significant changes as a consequence of the presence of the COVID-19 pandemic. The Government of Indonesia has instructed physical distancing so that teaching and learning activities, including in the Faculty of Medicine, must be carried out at home with online methods to reduce the risk of COVID distribution. This study aimed to explore undergraduate medical students' perspectives towards distance learning activities during the early period of COVID 19 pandemic at Muhammadiyah Surabaya University - Faculty of Medicine (MSU-FM), Indonesia. This study used a qualitative study design. The students' online survey responses had been done. Thematic analysis based on the Unified Theory of Acceptance and Usage of Technolgy (UTAUT) constructs was done to explore students' perspectives. As the results, students have a positive perspective related to the performance expectancy of several distance learning platforms towards distance learning activities. This factor has been perceived as a factor that support the behavior of the utilization of distance learning activities to increase cognitive involvement towards the whole learning process. However, in this study, other factors conveyed by students limiting their optimal distance learning experiences. In conclusion, students' user experiences must be developed and maintained continuously during this pandemic era. Longitudinal studies that investigate the long-term impact of various specific distance learning methods, assessments, and platforms' usage also needed.

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INTRODUCTION

Over the last six months, Covid-19 has relentlessly spread as a pandemic across the world, severely disrupting the lives of the three-quarters of the world population that reside within low- and middle-income countries (LMIC). These economically disadvantaged countries in Latin America, South East Asia, and Sub-Saharan Africa have not previously developed healthcare and medical education systems to respond to the current challenges presented by the pandemic effectively. The major challenge of the COVID-19 pandemic to all countries, including LMIC, has been to their healthcare systems. Before the COVID-19 pandemic in LMIC there was already the combination of high population health demand and insufficient resources, with low numbers of skilled health professionals and medical educators (Cecilio-Fernandes, Parisi, Santos, & Sandars, 2020). In response to the COVID-19 pandemic, most medical schools across the world, including LMIC, have started to rapidly transfer their curricula from face to face to online delivery using (McKimm, Gibbs, Bishop, & Jones, 2020).

Medical education throughout the world has experienced significant changes as a consequence of the presence of the COVID-19 pandemic. The COVID-19 pandemic requires educators around the world to rethink how they can continue to provide high-quality medical education at a time when medical school is in a situation of large-scale social restrictions. When considering the implementation of distance learning within a medical school or program, robust evidence-based research may strengthen one's position when encouraging faculty to remain abreast of technological advances. It will aid in addressing underlying concerns amongst medical faculty who may be resistant to integrating distance learning into teaching practices.

The Government of Indonesia has instructed physical distancing so that teaching and learning activities, including in the Faculty of Medicine, must be carried out at home with online methods to reduce the risk of COVID distribution. 19. Changes in this condition certainly have the potential to have an impact on general learning activities that were initially dominated by face-to-face methods. Therefore this study aims to explore undergraduate medical students' perspectives towards distance learning platform utilization during the early period COVID 19 pandemic at Muhammadiyah Surabaya University - Faculty of Medicine (MSU-FM), Indonesia. The results of this study can be used as a reference material for evaluating distance learning by other Faculties of Medicine & Health Sciences throughout Indonesia.

METHODS

This qualitative study was conducted from early to late July 2020 at the MSU-FM. The research questions to be explored are;

"What are students' perception towards distance learning activities at the Faculty of Medicine, University of Muhammadiyah Surabaya during early period COVID 19 pandemic era?"

The study population included all undergraduate medical students of the MSU-FM from first to final year, both male and female. Inclusion criteria of this study are:

1. Undergraduate medical students who are registered as active student.
2. Undergraduate medical students who are willing to be involved in this research.
3. Undergraduate medical students who are participated in distance learning during the Covid-19 pandemic.

The exclusion criteria of this study are students who are not willing to be involved in the research.

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A self-administered questionnaire was provided online to all the students' as a web link. The purpose of the study was explained to all the students and digital consent was obtained from all the participants. The survey's data collection period was done within 5 days. This study has been approved by the ethical review committee of MSU (Certificate number 019/ KET/II.3.AU/F/2020).

The existing students start from the class of 2016 to 2019. The total number of students in the MSU-FM is currently around 230 peoples. A number of 189 students completed the questionnaire voluntarily and anonymously. No other personal data other than the class-degree program, gender, students' ID number, and age were collected to avoid re-identifiability of individuals, which would otherwise have been possible because of the small cohorts. The questionnaire included open-ended questions regarding students' perceptions of distance learning activities during this pandemic situation.

Thematic analysis of the survey feedback was carried out in the following order;

a. Coding. Two researchers (ER & ALP) coding all the survey responses. Data, in the form of written testimonials from students that have been collected, were grouped based on gender (M=Male; F= Female), class-degree (A=2019 – D=2016), and time-based order of survey submission (number 1-100 for each class).

Formation of themes and sub-themes. Composition of themes and sub-themes were arranged deductively from the existing definition of factors in the Unified Theory of Usage & Acceptance of Technology (UTAUT) (Venkatesh, Thong, & Xu, 2016)

by two researchers (MRU & YL). Themes and sub-themes that emerge following the theoretical framework were analyzed more deeply in an iterative way to answer predetermined research questions through two sessions of online meetings among all researchers. The data processing software used in this research is Microsoft Excel 2016 and Atlas.ti.

RESULTS

Students have a positive perspective related to distance learning performance expectancy. This factor has been perceived as a factor that supports the behavior of the use of distance learning to increase cognitive involvement in the learning process. However, in this study, other factors such as; effort expectancy, social influences, facilitating conditions, intention to use, and previous experience conveyed by students limiting their optimal use of current distance learning platform.

The following themes were found from thematic analysis of undergraduate medical students' perspectives towards distance learning platform utilization during early 4 months period (March-June) of full distance learning activities at Muhammadiyah Surabaya University - Faculty of Medicine (MSU-FM);

Intention to utilize distance learning depends on quality of the learning media and previous learning experience.

Students' intention to use distance learning platforms (such as moodle-based e-learning, video conferencing, social networks, etc.) depends on the quality of the learning materials' design and format that had been provided on it. Students prefer optimized multimedia than regular text-only PowerPoint slides. The previous direct hands-on learning experience in the practice of medical skills has become a standard of satisfaction for students' psychomotor education activities. Meanwhile, the current online approach on learning medical skills which were delivered through distance learning at homes/ dormitories has brought difficulties, challenges, sense of doubt and uncertainty due to limitations of their training resources.

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"... for people like me who have problems with continuously changing learning styles/ method, the video becomes a safe enough learning resource to study medical topics, especially if the video content is made interesting." [MA11]

"I personally feel very dissatisfied with PowerPoin that had been given to us through the e-learning platform because it is difficult to understand, especially those PowerPoin that has a lot of pictures and abbreviations. Honestly, it is very confusing. My suggestion is that PowerPoin should be equipped with an explanation video or maybe enhanced by voice-over input on the PowerPoint. Maybe by that, we more interested in visiting e-learning more often" [MC07]

"For the skills lab, it is unfortunate because of the lack of adequate infrastructure or facilities in the home. Those obstacles make us hampered in the application of certain skill topics. It is because basically, the skills lab aims to train our' skills in the medical field, whereas, with the current situation, the lack of practice quality makes us confused and doubting its application in the real world. However, the skills lab' introduction lecture can still be carried out by providing online materials through recorded-demonstration video or PowerPoint with voice over or live streaming video conference" [FD24]

The performance expectancy of several distance learning platforms usages had positive influences on distance learning activities.

Students' were giving positive feedback about the utilization of several distance learning platforms. A few platforms were acknowledged as more convenient and suitable to be used when compared to features on a moodle-based e-learning platform.

"PowerPoint should be inputted with audio to explain difficult terms, charts, and images that are in it. There should still be Q & A sessions/ forums using recorded/ streamed videos other than Q&A forums in e-learning. The e-learning application we used today needs to be upgraded, so we don't need to refresh the web page continuously, especially if we want to update information"[MB05]

"For tutorials using video conferencing apps, it can help replace face-to-face meetings with tutors because what is expected from the tutorial is that students are able to have active discussions to find solutions to existing problems. So I think with the presence of video conferencing, you can still run the tutorial "[FC03]

"Online tutorials are useful. Using WhatsApp for the first meeting is more convenient, and the response is faster than using e-learning chats/ forums. During the second meeting, it was easier for us to understand the findings and discussion with the use of Webex / Zoom / other online meeting tools compared to the use of e-learning discussion forums "[MC11]

Effort expectancy of several distance learning platforms usages had less positive influences on distance learning activities.

Students' were giving less positive feedback towards the effort expectancy of several distance learning platforms utilization, especially when it comes to internet speed, bandwidth, and cost. A few platforms, which were recognized as more convenient and more suitable to facilitate learning activities when compared to the moodle-based e-learning platform, have been reported to be cost-dependent to get higher quality performance.

"Skills lab needs a recorded-video to explain detail practical steps. Because when using video conferencing platforms (Zoom / Webex) the problem is that the student's internet connection

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is not always good (so the information received at the session is interrupted, and learning becomes ineffective). Besides, not all students have WiFi. On the other hand, Zoom / Webex is very much draining student internet quota"[FD17]

"In carrying out the skills lab ... it needs a smooth connection while the WiFi connection at home is very unpredictable. So sometimes it is necessary to prepare an extra modem to backup the internet supply. "[FA30]

"... but internet signals in my area are difficult to obtain and there is no WiFi at home. I often have to reconnect when a tutorial or skills lab takes place."[MB12]

Facilitating conditions of independent learning, professionalism, & soft skills through distance learning activities were need to be improved

Students perceive formative assignments given in the form of quizzes as an ineffective additional learning burden in terms of duration and scheduling aspects. Some aspects of competence, such as professionalism, ethics, soft-skills, and empathy, are challenging to practice and develop in distance learning situations during this pandemic.

"... it is expected that the assignments can be reduced because by giving quite a lot of assignments and sometimes the collection deadline is very short, making students overwhelmed and more focused on the assignment compared to exploring the material independently after expert lecture takes place. The reality in the field many students are only stunned to search for answers that are makeshift with short time ... because each student has a different pace and style." [FC14]

"... reduce the number of daily quizzes, especially those that require us to work late, because sometimes we have to prioritize these quizzes rather than to study independently ... I have to pay attention to my sleep during this pandemic."[MA07]

"... the professionalism, confidence, ethics, and empathy cannot be seen and taught optimally in the current conditions."[MD06]

Social influences were inhibiting the optimum usage of some platforms on distance learning activities.

Students' were reporting their adjustment towards shifting into an informal learning environment. Some of them felt their current informal learning environment not yet conducive for them to achieve optimal study.

"Online tutorials using either Webex or zoom can indeed help, but not every individual can focus on online learning when they are at home or not face to face directly, many have seen in some of my friends."[FB18]

"... for example, on the topic of history taking, probandus must understand the contents of the scenario, the family/people in the house/ boarding house will not be competent in this matter"[MA02]

DISCUSSION

In general, distance learning in the context of health professional education has been known to produce positive academic outcomes (Bernard, Borokhovski, Schmid, Tamim, & Abrami, 2014; De Leeuw, Walsh, Westerman, & Scheele, 2018; Fawns, Jones, & Aitken, 2020; Pusponegoro, Soebadi, & Surya, 2015; Taylor et al., 2020)

Six months since the pandemic begin, students' intention towards distance learning and the use of distance learning platforms at MSU-FM still needed to be developed. Attitude and behavioral shifting from just fulfilling academic obligations into independent intentions to optimally use distance learning must be directed and maintained continuously

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during this early adaptation phase. Improving the quality of learning media and engagement towards daily learning activities as needed in order tho achieved that shift.

During this fully online shifting period, our school has been faced with several challenges. Our first challenge was the difficulty in monitoring student engagement. Any online video-conferencing program has its limits on the maximum number of participants that could be viewed on a single screen, and we were unable to see the whole class at a glance. Even if the student is visible on the video-conferencing program, the ability to determine the student's focused attention and full engagement remained elusive. The lack of clear visibility on students' learning behavior could also challenge faculty's ability to monitor student's wellbeing and professionalism, especially in assessment activities.

Our second challenge was that internet connectivity and technological literacy problems hindered the smooth implementation of online courses. Online courses (especially tutorials, skills practices, and any other form of psychomotor activity) in our institution are heavily dependent on the use of multiple technologies and platforms. These include video-conferencing tools, test-taking platforms, and a live shared document for students to discuss pertinent topics to the session. Participants (students and faculty) are expected to have a degree of proficiency in the use of these platforms. The rapid transitioning to online learning made it challenging to ensure all participants were fully prepared beforehand.

Those challenges were coherent with findings from intention to use, previous experiences, performance expectancy, and effort expectancy theme, which has led to the need of our students' on the usage of other distance learning platforms or social media as a learning platform. These findings also emphasized the importance of ethical education and professionalism in the digital learning environment for medical students, which were also stated in facilitating conditions theme. Previous studies before the pandemic situation also acknowledge that the use of those supporting media should also be balanced by the improvement of students' and facilitators' digital literacy skills (O'Doherty et al., 2018; Thorell, Fridorff-Jens, Lassen, Lange, & Kayser, 2015). Related to this, several skills that need to be developed including; productive and constructive behavior in providing feedback at the digital environment, the ability to manage information and feedback in a professional manner, and the development of professional identity from the virtual community of practice (O'Doherty et al., 2018; Mesko et al., 2015; Siddiq et al., 2017; O ,Regan et al., 2018).

Facilitating conditions are things that need to be developed in detail by instructors and instructional design developers. Although learning content has been given on schedule, several aspects such as the balance of the duration of online and off-line quizzes as well as the lack of quality and quantity of multimedia teaching materials, have been reported by students in this study as obstacles to achieving optimal learning outcomes. Thus, the need for a balanced schedule of quizzes/ games (gamification) to optimizing effective spaced-repetition is needed and must be designed contextually based on students' characteristics, lecturers, and learning objectives (Cook, Levinson, & Garside, 2010; Lau, 2014)

The utilization of other distance learning platforms/ social media/ third party applications is also needed to be developed and must be monitored through rigorous learning analytics (McKimm et al., 2020; Samarasekera et al., 2020; Tang et al., 2018; Utama, Yuliawan, Suhoyo, & Doni, 2020).

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In the face of this substantial disruption in the clinical and academic learning environments, Hall et al. (2020) stated that students would undoubtedly require an increase in attention to individualized learning plans and longitudinal coaching. Engaging with coaches or academic advisors can help programs to detect impending or active problems in current learning environments or situations and then enhance the situation to find adaptations or solutions. The core features of a coaching relationship include a shared orientation towards growth and development, ongoing reflection, and an embrace of failure (or difficulty in our case) as a catalyst for learning. Engaging with a coach or academic advisor (virtually or in-person) to generate an effective individualized learning plan can help learners focus on pursuing key potential learning activities and acquiring necessary assessments rather than struggling with the difficulties. This may mean more frequent meetings and regular check-ins with program directors, academic advisors, or other assigned coach/mentor figures to jointly ensure trainees are on the right track (Hall et al., 2020)

Factors affecting students‘ and facilitators‘ resilience towards daily learning activity, which were affected by the pandemic situation also should be considered and maintained carefully. Resilience in the health professional depends on the dynamic process of interaction between individual factors, environment, and coping strategies, as well as deliberate interventions to improve one’s resilience (Huey & Palaganas, 2020).

Technical problems, domestic obligations, changing daily routines, and many other forms of behavioral adaptation would become barriers to achieve optimal positive adaptation. Several interventions by school management system such; mindfulness programs, work/ study-life balance activity programs, resiliency workshops, and many other forms could be adapted to enhance resiliency and engagement towards daily learning activities (Huey & Palaganas, 2020; Kangas-Dick & O’Shaughnessy, 2020).

Our program anticipates that the disruption caused by COVID-19 will eventually lessen or end. It is important that we have to prepare for this post-pandemic period of education and clinical catch up with a plan to help and support students in their quest to be competent and independent practitioners. This will be the time to refocus on medical education. During the pandemic disruption, students may have missed or had variable training experiences. Our Medical Education Unit already plans several movements to assure readiness for the post-pandemic period. Enhance and maintain students’ and faculties’ engagement/ empowerment along this process will be key as priorities during this time of disruption. A view of those plans are;

1) Periodically deploy a survey to update socio-economical and health status of each students & faculties;
2) Periodically deploy a survey to update students’ and faculties’ facilitiating learning needs & experiences;
3) Complete a detailed review of each student’s progress in attaining competencies and training experiences through students’ digital portfolio;
4) Revamp and updated the schedule both broadly and individually for students together with academic coach and program’s directors;
5) Develop a student support sub-unit which puts attention on student’s customized coaching or mentoring needs and also collaborate with them in organizing self-development/ improvement online sessions.

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It is also important for programs to anticipate the ongoing impact of COVID-19. There will be pressure to manage the immense backlog of practical and medical skill achievements or comprehensive procedural-related assessments caused by the pandemic. There may also be increasing pressure to limit student involvement in procedures in the interest of efficiency. In this situation, every program will need to consider innovative ways to maintain casual training while balancing economic demands to ensure the acquisition of competencies. For example, we may use student-specific learning goals based on entrustable professional activities to direct a student's involvement in a procedure to a specific part of the task. Programs should also explore additional possible training sites (including on community-based/ family practices education) to avoid student crowding and improve individual training experiences.

Finally, with its own unique contextual characteristics, there are opportunities to collaborate and share resources from each diverse undergraduate medical program in Indonesia. Inter-regional featured-lecturer, international guest speaker/ panelist, shared-digital learning resources/ repositories through open resource facilities, national/ international massive open-online courses which approved/ recognized as electives curriculum, etc. are few ideas of collaboration movements. By these movements, students' learning experiences could be enriched through shared-resources, shared-experiences, and virtual or digital collaborative learning activities among various medical programs in the midst of current limitation.

CONCLUSION

Most studies around distance learning in Indonesia only focus on one learning module/ topic or restricted by the contextuality of one medical program/ faculty. Therefore, generalization becomes a problem in itself. The researcher recommends implementing large-scale implementation studies from other educational institutions to look for evidence of generalization.

Longitudinal studies that investigate the long-term impact of using distance learning also need to be conducted. However, if all learning programs are redesigned by integrating flipped-classroom/ blended-learning with the utilization of various distance learning platforms for each block/ module, then the adequacy of the time allocation to adequately prepare themselves from the instructors, students, and all stakeholders involved needs to be evaluated. Besides, to achieve this, longitudinal assessment techniques that are able to measure the dynamics of changes in clinical reasoning, the level of cognitive engagement, and changes in behavior towards the learning process in the digital learning environment need to be developed first. In addition, issues surrounding the validity and reliability of online exams need to be prepared and evaluated.

The use of various learning methods, other distance learning platforms, usage of social media as main/ supporting media, and third-party applications for learning analytics, both separately and as a combination of components in the digital learning environment, needs to be explored continuously. By using quasi-experimental designs, a comparative study between all of them can identify the effectiveness and impact of continuously growing learning methods and media on medical students' outcomes.

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Case report

OVERLAPPING PRIMARY AND SECONDARY SYPHILIS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) PATIENT

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Abstract

Coinfection between syphilis and Human Immunodeficiency Virus (HIV) could have varied clinical manifestations. Overlapping of syphilis stages is accounted for 25% of cases, attributable to the defect of the cellular and humoral immune response. We reported 55 years old man who was sexually active since 25 years ago via genito-genital, genito-anal, genito-oral routes, had multiple sexual partners both male and female, seldom used condom, and always being the insertive. Dermatological examination revealed alopecia non-scarring, a painless solitary ulcer on the collum penis, also multiple erythematous macules, patches, and plaques all over the body. *Spirochaeta sp.* was found from the base of the ulcer with a darkfield microscope. Histopathology examination revealed secondary syphilis lesion. Venereal Disease Research Laboratory (VDRL) 1:16, Treponema Pallidum Haemagglutination test (TPHA) reactive, HIV Determine rapid test reactive, and CD4 (Cluster of Differentiation 4) T-cell count 111 cells/ µL. He was treated with a single dose of benzathine penicillin G 2,4 million units intramuscular and antiretroviral drugs. On sixth month evaluation, VDRL was non-reactive, and CD4 T-cell count 325 cells/µL. This case shows that overlapping clinical manifestations of primary and secondary syphilis on HIV patients could occur with a good general condition. Immediate treatment of antibiotics for syphilis and antiretroviral could improve the clinical and serological conditions.

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INTRODUCTION

Syphilis is a systemic disease caused by *Treponema pallidum* infection. This bacterial infection has a close relationship with Human Immunodeficiency Virus (HIV); both could be transmitted through sexual intercourse, often having coinfection, and could affect one another. World Health Organization (WHO) estimates there are 12 million new cases of syphilis every year, and 90% are in developing nations (Karp et al. 2009). While the prevalence of people with HIV in Indonesia was 0,4% of all adult population in 2018, that is as much as 640.000 (550.000 – 750.000) cases (UNAIDS 2020).

Syphilis could increase the risk of HIV transmission about 3-5 times higher (Pisani et al. 2004). Primary syphilis lesions on genitalia could be the HIV portal of entry and facilitate the transmission. People with HIV/AIDS are eight times more often to have positive syphilis serologic tests. Beside, syphilis could also increase the infection capacity of HIV. Syphilis seroconversion on HIV patients closely related to multiple sexual partners, drugs, struck up on the internet, and serosorting habit. The course of syphilis has a staging of clinical manifestation (primary stage, secondary stage, and tertiary stage) and asymptomatic or latent stage (Lukehart 2008). The course of infection on HIV, generally more severe, even more often, are asymptomatically. One of four syphilis and HIV patient having a primary and secondary lesion appear concurrently (Karp et al. 2009). HIV infection could give rise to varied clinical manifestations because of cellular immunity defect (Carlson et al. 2011). This paper will be reported in one case of bisexuals with overlapping primary and secondary syphilis and HIV.

CASE REPORT

A 55-years-old man came to dermatology and venereology outpatient clinic dr. Saiful Anwar Regional General Hospital (RSSA) complaining red rashes all over his body for three weeks before consulted. No complaint of itchy, pain, burn, nor numbness sensation on the rashes. His hair was starting to fell off since the appearance of outbreaks. He also complains of a wound on his genitalia for two days before consulted, which was painless, odorless, not even producing any secret. There was a history of mild fever, weakness, lethargy, decreased arousal and appetite, and decreased body weight for seven months before consulted. He was diagnosed with HIV by internist from private hospital two days before asked to a dermatologist and given antiretroviral therapy.

Currently, the patient works as an entrepreneur and has a boarding house business. He was never married. He had a sexual history since 25 years ago with male boyfriends, multiple partners, through a genital-anal and a genital-oral sexual course, he instead on “top” position, and never used condoms. Complaints on partners were denied. He also had sexual intercourse with female sex workers (FSW), through genital-genital or oral-genital sexual courses, rarely used condoms, complaints on sexual partners unknown. The last history of sexual intercourse was seven months ago with male boyfriend, through a genital-anal or a genital-oral sexual course, the patient rather on “top” position, did not use condoms. Complaints on the partner are denied.

His vital sign was normal, without any enlargement of lymph nodes. Dermatological examination revealed alopecia on the frontoparietal region (Figure 1A), a hair-pull test was negative. No abnormalities were found in the oral cavity. Scattered on the scalp, face, body, arms, legs, and also palms and soles, there

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were multiple erythematous macules, patches, and plaques with indistinct boundaries, varied in shape and size with an average diameter of <2 cm, non-confluent, and diascopy non-branch able (Figure 1B). On the penile shaft, there were solitary ulcers, round shape, with a diameter of about 1 cm, distinct border, not curled, the base is clean, no pain with touch or pressure is obtained (Figure 1C).

We revealed *Spirochaeta sp.* from the base of the ulcer (Figure 2A). A punch biopsy was taken from the erythematous plaque then processed and stained with Hematoxylin and Eosin (H & E), appearing thickened epidermis, marked acanthosis, dyskeratosis, and vacuolar degeneration. In the dermis, there appears to be a group of lymphocyte cells and round nucleated plasma cells with eccentric nuclei forming perivascular and periadnexal granulomas, dilated blood vessels and endothelial swelling like a cobblestone (figure 2B & 2C). Laboratory tests are shown in Table 1.

The patient was diagnosed with overlapping primary and secondary syphilis and HIV and treated with Benzathine Penicillin G 2.4 million units single-dose intramuscular and antiretroviral therapy of nevirapine 200 mg twice a day and a combination of lamivudine 150 mg and zidovudine 300 mg in the capsules twice a day. There were Jarisch-Herxheimer reactions and were controlled with the administration of Paracetamol 1500 mg dividing dose. We educated patients regarding the disease, to loyal to one partner, and to use condoms when having sexual intercourse.

Reddish rashes all over his body gradually became hyperpigmented a few days after therapy. The genital ulcer was healed 2 days after therapy. At the first and third month evaluation, there were no complaints from the patient. Dermatological examination revealed alopecia on the frontoparietal scalp with a negative hair-pull test (Figure 1D). No abnormalities in the genitalia nor throughout the body (Figure 1E). Laboratory tests are shown in Table 1.

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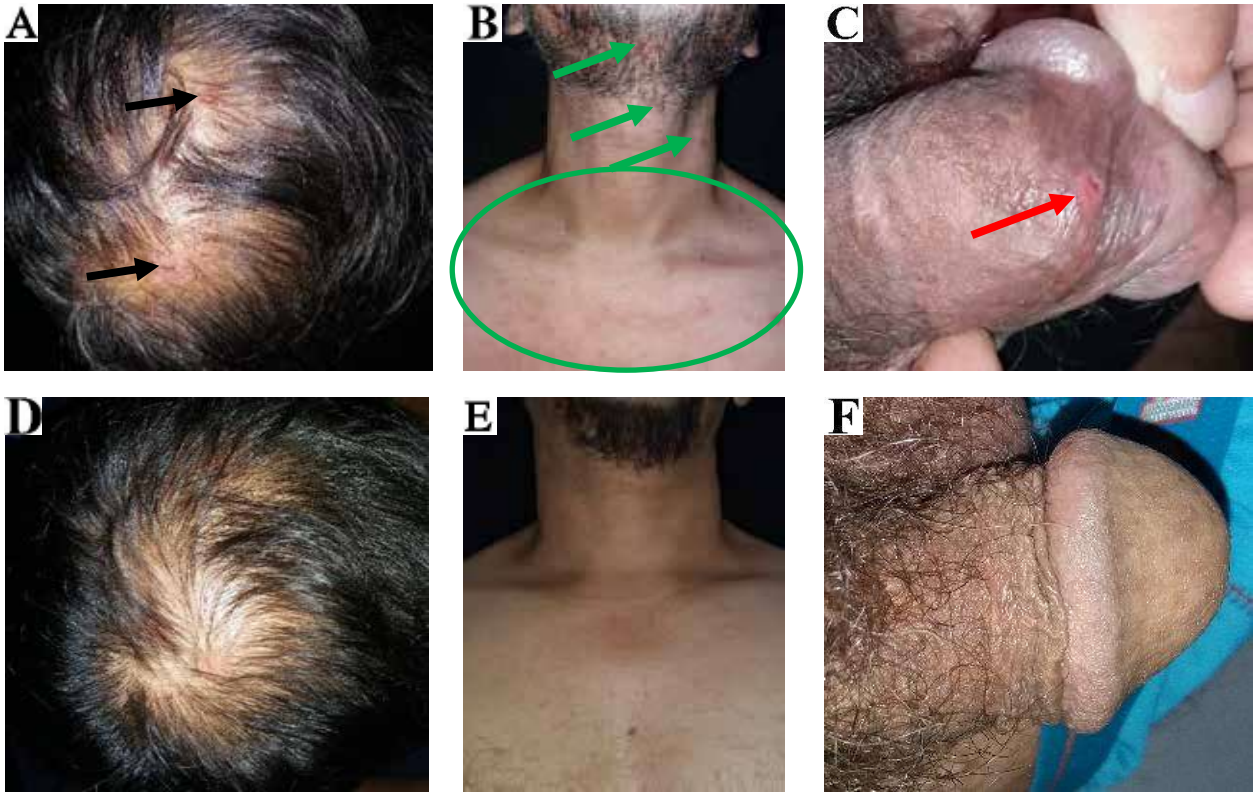

Figure 1. Dermatological examination at first visite (A-C) and 6th-month evaluation (D-F). A. On frontoparietal, there were alopecia non-scarring with multiple erythematous papules (black arrows). B. on the neck, there were multiple discrete erythematous macules and papules, representing the same lesion all over the body (green marking). C. On the collum penis, there were shallow ulcers, a distinct border, and a clean base (red arrow). D. Alopecia without any other rash. E-F. No rash all over body and genitalia.

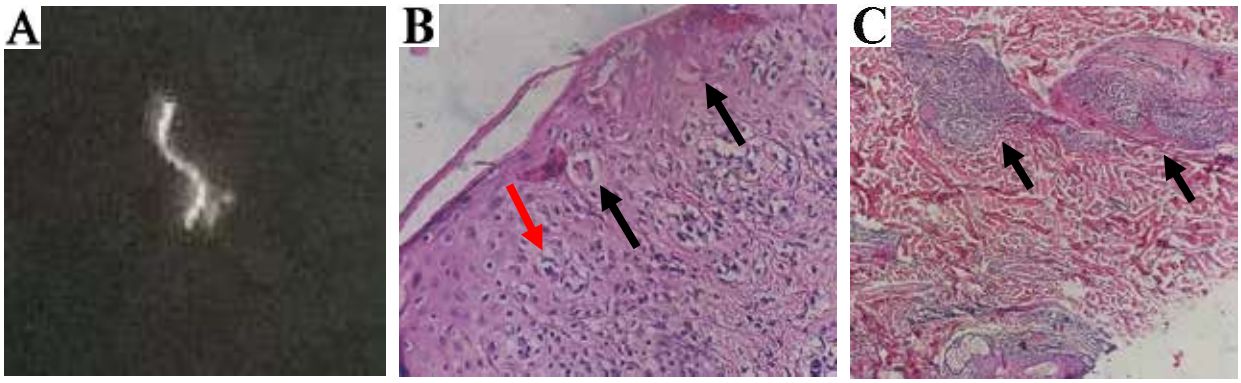

Figure 2. Laboratory examination. A. A *Spirochaeta sp.* (darkfield microscope, x200). B-C. Histopathological examination, H&E. B. The thickened epidermis, marked acanthosis, dyskeratosis (black arrows), and vacuolar degeneration (red arrow) (x400). C. A group of lymphocyte cells and round nucleated plasma cells with eccentric nuclei forming perivascular and periadnexal granulomas (black arrows), dilated blood vessels and endothelial swelling like a cobblestone (x400).

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Table 1. Laboratory examination

Month	VDRL	TPHA	CD4 T-cell count (cells/μL)
0	1:16	Reactive	111
1	1:4	Reactive	-
3	1:4	Reactive	314
6	Non-reactive	Reactive	325

VDRL, Veneral Disease Research Laboratory. TPHA, Treponema Pallidum Haemagglutination test.

DISCUSSION

Syphilis epidemiology had been raised for years since the beginning of this century, and HIV likewise. This is consistent with the changing of sexually risky behavior (Pisani et al. 2004; Solomon and Mayer 2015). Even though the data about syphilis and HIV incidence in Asian countries is limited and most reporting syphilis prevalence regardless HIV status, some study report the prevalence of syphilis and HIV are higher in the key population such as men who have sex with men (MSM), transgender, and male/female sex worker (MSW/FSW) (Ahn et al. 2016; Daili et al. 2013).

Syphilis and HIV infections have a synergistic negative effect (strengthening each other). Both have effects on cellular and humoral immunity. Biologically, primary syphilis could increase transmission of HIV; epithelial and mucosal surfaces damage becomes a portal of entry for the virus to reach the systemic circulation. Genital mucosal damage caused by the bacteria could facilitate the transmission of HIV virion through the recruitment of inflammatory cells or increase the number of receptors expressed by each cell. Dendritic cells that express the CD4 receptor and CCR5 (C-C chemokine receptor type 5) are the main targets of HIV-1. Macrophage-tropic (M-tropic) strains, the most infectious HIV strains frequently transmitted sexually, use CCR5 as a co-receptor for viruses. Meanwhile, HIV-1 T-tropic cells use the chemokine receptor, CXCR4 (C-X-C chemokine receptor type 4), and replicate in T-cells. *Treponema pallidum* lipoprotein can increase HIV-1 replication by inducing the expression of the HIV-1 gene in human monocytes via NF-κβ-dependent mechanism. Humoral response to HIV infection is secondary activation of polyclonal B-cells caused by CD8 T-cells suppression, this results in an increase in immunoglobulin levels, and the decrease in serological syphilis titers will be longer than expected. The second response is the activation of suppressed B-cells so that there is a decrease in response to mitogens or antigens, causing false negatives on serological tests (Carlson et al. 2011).

In the presence of HIV coinfection, the clinical manifestations that determine the stages of syphilis can vary, and each stage can overlap (Table 2). These variations might be due to a defect in humoral and cellular immunity. Qualitative changes in the immune system in HIV patients can cause acceleration of the appearance of secondary lesions, more atypical features, more extensive and slow healing of primary lesions. Syphilis and HIV patients more often manifest as secondary syphilis compared to patients without HIV, and chancre coexistence can occur. Also, they are more likely to develop into neurosyphilis, which appears clinically (Carlson et al. 2011; Hutchinson et al. 1994). Simultaneous clinical manifestations of primary and secondary syphilis can occur in 25% of HIV coinfected

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patients (Szetela and Gasiorowski 2016). The tendency of this disseminated syphilis is usually increased with the number of CD4 T-cells of less than 200 cells/µL (Jackson and Price 2013).

Alopecia in the frontoparietal region of the patient that occurred in the last seven months, indicates hair loss. A simple method for evaluating hair loss is a hair pull test. Three hairs are removed from the entire head area with a hair bulb so that patients are suspected of having non-scarring alopecia (Jackson and Price 2013). Alopecia non-inflammatory and non-scarring could be caused by syphilis infection, though it is rare. This type of alopecia should be differentiated with alopecia areata, trichotillomania, traction alopecia, and alopecia neoplastic, which can all be ruled out by biopsy (de Sousa 2013).

Most guidelines and experts agree that serological tests for syphilis can be interpreted the same in patients with or without HIV infection. If the titer is deemed inappropriate, a serological test and direct method (dark field microscope and/or painting of Warthin-Starry silver or fluorescent antibody on biopsy tissue) can be performed (Frieden et al. 2015; Rowawi, Djayakusumah, and Achdiat 2018). Besides, patients with syphilis and HIV who have a higher risk of neurosyphilis are still unclear. Guidelines from the European Union and the USA recommend a pre-therapy cerebrospinal fluid examination in advanced latent syphilis or unknown duration of HIV if the CD4 T-cell count is less than 350 cells/mm^3 and the RPR (Rapid plasma reagin) titer exceeds 1:32 (Frieden et al. 2015).

The therapeutic recommendation for early and advanced syphilis is benzathine penicillin G; it has a long half-life, appropriate with the 30-hours-division of *T. pallidum*, and not toxic. The regimen is the same as in patients without HIV because no syphilis therapy regimen was more effective for preventing neurosyphilis in HIV patients. The recommended therapeutic regimen is benzathine penicillin G 2.4 million unit single-dose intramuscular for primary and secondary syphilis with HIV. The use of antiretroviral therapy, according to HIV guidelines, is recommended in these patients. The addition of benzathine penicillin G doses or other antibiotics, such as amoxicillin, in primary and secondary syphilis did not show significant differences (Frieden et al. 2015).

Table 2. Atypical features of syphilis in the HIV-seropositive patients

- Higher rate of symptomless primary syphilis.
- Primary syphilis with multiple or deeper chancres.
- Higher rate of secondary disease.
- Overlap of primary and secondary stage features of syphilis.
- Increased rate of early neurologic and ophthalmic involvement.
- More rapid progression to tertiary manifestation.
- Report of false-negative serology in both primary and, less commonly, in secondary syphilis.
- Reduced efficacy of standard therapy for early syphilis.
- Relapse is more frequent.
- Jarisch-Hersheimer reaction is more frequent.
- Normalization of CSF values after treatment: delayed.
- Prozone phenomenon more frequent.

HIV: Human Immunodeficiency Virus
Source: Pialoux et al. 2008

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Treatment with a high dose of penicillin can cause side effects such as the Jarisch-Herxheimer reaction and anaphylactic shock. The Jarisch-Herxheimer reaction is the reaction of acute fever followed by headache, myalgia, fever, and other symptoms that occur during the first 24 hours after syphilis therapy. This reaction is not a hypersensitivity reaction, but rather the presence of cytokines triggered by the dead *T. pallidum* lipoprotein, so penicillin therapy does not need to be stopped, and antipyretics can be used to treat symptoms (Rowawi, Djayakusumah, and Achdiat 2018).

VDRL titers should drop four times fold at three months, eight times fold at six months, and be negative in 1 year for primary syphilis and two years for secondary syphilis. The VDRL titer of our patient became non-reactive after six months. Smith in 1950 shows that 21% of syphilis and HIV patients treated with benzathine penicillin G for primary and secondary syphilis were getting relapse after 18 months while 3 out of 4 patients with secondary syphilis and HIV did not recover. It should be noted that the serological response to therapy are more unpredictable in patients with HIV but does not indicate a worse clinical response (Carlson et al. 2011). Clinical and serological follow-up of treatment was carried out at 3rd, 6th, 9th, 12th, and 24th months after therapy. If at any time, clinical symptoms reappear or persist, or nontreponemal titers remain or increase, cerebrospinal fluid examination and repeat therapy should be carried out (Rowawi, Djayakusumah, and Achdiat 2018).

CONCLUSION

We reported one case with a diagnosis of overlapping primary syphilis and secondary syphilis and HIV in 55-year-old male patients with a good general condition. Diagnosis is based on history, physical examination, dark field microscope, and histopathological examination. Patients were given therapy for benzathine penicillin G 2.4 million units single-dose intramuscular and antiretroviral therapy. After given antibiotic and antiretroviral therapy, clinical conditions are improved and no manifestations of primary, secondary, nor tertiary syphilis. At the 3rd month evaluation, there were four times the fold decrease in VDRL titers and become non-reactive in the 6th month. Patients will continue to be followed up at 9th, 12th, and 24th months for VDRL titers. Patients are advised to be loyal to one partner and use a condom when having sex.

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