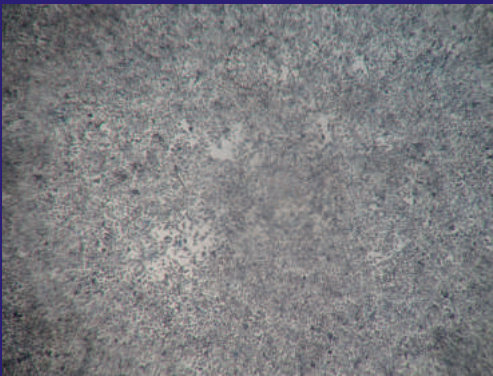


Indonesian Journal of Tropical and Infectious Disease



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Indonesian Journal of Tropical and Infectious Disease

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Review Article

Histoplasmosis: Diagnostic and Therapeutic Aspect

Meiliyana Wijaya^{1,3}, Robiatul Adawiyah², Retno Wahyuningsih^{2,4*}

¹Study Program of Clinical Parasitology, Department of Parasitology, Faculty of Medicine, Universitas Indonesia

²Department of Parasitology, Faculty of Medicine, Universitas Indonesia Jakarta, Indonesia

³Department of Parasitology, Faculty of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia.

⁴Department of Parasitology Faculty of Medicine, Universitas Kristen Indonesia Jakarta, Indonesia

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ABSTRACT

*Histoplasmosis has been reported since 1932 in various regions in Indonesia. This disease is caused by thermally dimorphic fungus *Histoplasma capsulatum* var. *capsulatum* which is experiencing an increasing incidence worldwide. Human infection occurs when spores in soil contaminated with bird and bat droppings are inhaled and change to form yeast in the lungs. The majority of these forms of infection are mild and can heal on their own, but if large numbers of spores/inoculum are inhaled, or the host is immunosuppressed, serious lung disease and even dissemination may occur with a high mortality rate. The diagnosis can be made by combining clinical symptoms with laboratory test results. Conventional laboratory methods such as direct examination or histopathology and culture are the gold standards for histoplasmosis diagnosis. The weakness of culture is the nature of *H. capsulatum* as a slow grower fungus that takes 4-6 weeks to grow. In addition, laboratory tests can be carried out with antibody detection or antigen detection. Antigen detection is more beneficial for hosts with immunosuppression or acute form, while antibody detection is more important in the chronic form of the diseases. Molecular-based assays have high specificity but are not yet available commercially and are more widely used for culture identification to confirm the species of *H. capsulatum*. Histoplasmosis therapy usually begins with the administration of amphotericin B for around two weeks, followed by maintenance with itraconazole for 6 - 9 months duration. A careful history of possible exposure and the appropriate laboratory diagnostic approach is essential to provide appropriate therapy.*

Keywords: *Histoplasma capsulatum*; pulmonary; dissemination; laboratory diagnosis; antifungal.

ABSTRAK

*Histoplasmosis telah dilaporkan sejak lama di berbagai daerah di Indonesia yakni sejak tahun 1932. Penyakit ini disebabkan oleh jamur dimorfik bergantung suhu *Histoplasma capsulatum* var. *capsulatum*. Histoplasmosis saat ini tengah mengalami peningkatan kejadian di berbagai penjuru dunia. Infeksi pada manusia terjadi ketika spora yang terdapat di tanah yang dicemari oleh kotoran burung dan kelelawar terhirup ke saluran napas dan berubah menjadi bentuk ragi di paru-paru. Pada umumnya bentuk infeksi ini bersifat ringan dan dapat sembuh spontan, namun bila spora/ inokulum yang terhirup berjumlah besar atau pejamu dalam kondisi imunopresi akan dapat terjadi penyakit paru serius bahkan diseminasi ke seluruh tubuh dengan angka kematian yang tinggi. Diagnosis histoplasmosis dapat ditegakkan dengan menggabungkan gejala klinis dan hasil pemeriksaan laboratorium. Metode laboratorium konvensional seperti pemeriksaan langsung atau histopatologi, dan kultur adalah baku emas untuk diagnosis histoplasmosis. Kelemahan kultur terletak pada lamanya waktu yang diperlukan untuk tumbuh sempurna agar dapat diidentifikasi secara morfologis yakni mencapai 4-6 minggu. Selain itu, pemeriksaan laboratorium juga dapat dilakukan dengan deteksi antibodi atau deteksi antigen. Deteksi antigen lebih bermanfaat untuk pejamu imunopresi atau bentuk akut, sementara deteksi antibodi lebih bermanfaat untuk histoplasmosis kronik. Pemeriksaan berbasis molekular memiliki spesifisitas tinggi namun belum tersedia kit komersial untuk penggunaan rutin dan lebih banyak digunakan untuk memastikan spesies. Terapi histoplasmosis biasanya*

* Corresponding Author:
retnet@hotmail.com

dimulai dengan pemberian amfoterisin B selama kurang lebih dua minggu dilanjutkan terapi rumatan dengan itrakonazol selama 6-9 bulan. Anamnesis yang cermat tentang kemungkinan riwayat pajanan dan pendekatan diagnostik laboratorium yang tepat sangat penting untuk menegakkan diagnosis yang akurat sehingga dapat diberikan terapi yang tepat.

Kata kunci: *Histoplasma capsulatum*; paru-paru; diseminata; diagnosis laboratorium; antijamur.

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INTRODUCTION

Histoplasmosis was first found in 1905 by a pathologist named Samuel Taylor Darling, who autopsied an African carpenter's body with irregular fever, weight loss, splenomegaly, leukopenia, and anemia previously. On autopsy, the cause of death initially was suspected due to tuberculosis (TB). Nevertheless, smears from the patient's granulomas in the lung, bone marrow, liver, and spleen revealed no TB bacilli. Then Darling suggested that the microorganism cause the disease was a protozoan and named it *Histoplasma capsulate* (now *Histoplasma capsulatum*).¹ In 1912, Henrique da Rocha-Lima compared the findings of Darling's slides with fungal of *Cryptococcus* and concluded *Histoplasma capsulatum* (Hc) was a species of fungus rather than a protozoan then classified into the kingdom Fungi with phylum Ascomycota until now.^{2,3}

The causative species comprised three taxonomic varieties. Two distinct pathogenic varieties for humans are *H. capsulatum* var. *capsulatum* (Hcc) and *H. capsulatum* var. *duboisii* (Hcd), whereas *H. capsulatum* var. *farciminosum* is pathogenic in horses.⁴⁻⁶ In this review, we will focus on histoplasmosis due to Hcc, which distributed more widely.

The infection due to Hc occurred when spores located in polluted soil contaminate the surrounding air then inhaled and transformed into yeasts in the lungs.^{7,8} The fungus' primary habitat is soil contaminated with bird or bat excreta and chicken droppings. Excrement from several avian species has been incriminated in

enhancing the fungus' growth due to nitrogen/phosphate-enriched soil.^{3,7,9}

The Hc infection can be the source of symptoms in immunocompromised and immunocompetent patients.^{10,11} The clinical spectrum of disease can be severe and mortality high due to its ability to disseminate.¹² Hence, histoplasmosis became a significant public health problem since the AIDS pandemic era and more widely known with other immunosuppressed conditions.¹³⁻¹⁵ Herein, we review histoplasmosis diagnosis from clinical features and laboratory diagnostic methods, with a therapeutic approach depends on its clinical finding.

EPIDEMIOLOGY

Indonesia's first case of histoplasmosis was first noted by Mueller in East Java, Indonesia, in 1932.¹⁶ Histoplasmin skin test (HST) methods previously suggested for diagnosis of histoplasmosis in immunocompetent individuals. This test can detect if people have been exposed to Hc, which is similar to the tuberculin test that can detect *Mycobacterium tuberculosis* exposure. Nonetheless, HST is currently more widely used in epidemiological studies to determine histoplasmosis's endemicity, not to diagnose histoplasmosis.^{17,18} Historically, Indonesia has done the HST in Jakarta, Surabaya, Bali, and Medan in the 1900s with positive results ranging from 12,5-63.6%.¹⁹ Since then, sporadic cases of histoplasmosis have also been reported from various other regions in Indonesia (Java, Sumatera, and Sulawesi).¹⁹⁻²²

CLINICAL FEATURES

In immunocompetent hosts, most patients are self-limited mild pulmonary infections remarkably never recognized as being histoplasmosis.^{23,24} However, in immunocompromised patients or extreme of age or ensuing inhalation, a large inoculum typically presents as a pulmonary disease with diverse manifestations. Those features including acute pulmonary histoplasmosis, chronic cavitary pulmonary histoplasmosis, a complication of pulmonary histoplasmosis, and progressive disseminated histoplasmosis (PDH).^{25–27}

Symptomatic acute pulmonary histoplasmosis is a self-limited disease and often presents in children or adolescents when exposed to the environment's organisms. Symptoms of flu-like syndromes consist of fever, malaise, dyspnea, dry cough, and pleuritic chest pain arising at a median of two weeks after exposure. Chest radiographs show diffuse bilateral patchy infiltrates, while chest computed tomography (CT) frequently shows enlarged hilar lymph nodes or mediastinal. A careful history of Hc's possible inhalation is essential to arrive at the appropriate diagnosis due to similarity with community-acquired bacterial and viral pneumonia. Acute severe pulmonary infection occurs when the host is immunocompromised or inhale a large inoculum of Hc conidia. Patients with this condition and who have severe dyspnoea may develop acute respiratory distress syndrome rapidly.^{24,27,28} Infection may also be subacute, which evolves slowly over several weeks after exposure to smaller inocula. Subacute manifestations may present with milder symptoms yet more persist with respiratory and constitutional symptoms e.g., cough, fever, and malaise that last several weeks to a month.^{26,27}

The manifestation of chronic cavitary pulmonary histoplasmosis occurs primarily in the elderly with underlying structural pulmonary disease or smoking history. The previous history of pulmonary disorders (e.g., emphysema) causing disturbance of Hc clearance, resulting in long-lasting latent inflammation occurs adjacent to preexisting

bullae, leading to large cavitary lesions.^{23,26,27} Clinical manifestations that can occur include chronic productive cough, hemoptysis, dyspnea, chest pain, nonspecific fever, night sweats, fatigue, and weight loss. These clinical symptoms can take months to years. Chest imaging may demonstrate focal or diffuse infiltrates, nodules, consolidations, and cavitation, commonly in the lung's apical region. In the late phase, we may see interstitial fibrosis and pleural thickening. Clinical and chest imaging features resemble other chronically destructive pneumonia, including TB and other pulmonary fungal infections like chronic pulmonary aspergillosis.^{27,29} In Indonesia, where TB is endemic, the differential diagnosis of histoplasmosis should be considered, especially in multidrug-resistant TB. The progression of the chronic form is slow but can be fatal if left untreated.^{23,26,27,30}

Sequelae of pulmonary histoplasmosis mostly are asymptomatic calcifications and calcified mediastinal/ peribronchial lymph nodes. Nonetheless, progressive complication leads to obstructive symptomatology, including mediastinal granuloma, mediastinal fibrosis, and broncholithiasis can occur though are rare. Other uncommon manifestations seen in sequelae histoplasmosis patients include: pericarditis, arthralgias, erythema nodosum, and erythema multiforme.^{24,27}

The spectrum of PDH more likely to occur in the immunocompromised population. Before the frequent use of immunosuppressive therapy, PDH was seen predominantly in infants. Currently, most cases of PDH were reported in HIV-infected patients, especially with CD4 cells <150/ μ L.²³ Other immunocompromised patients are at risk of developing PDH, including those with the recipient of organ transplantation, hematologic malignancies, and long-term immunosuppressive therapy.³¹ Nonetheless, PDH has also been described in immunocompetent patients, apparently due to extensive inocula exposure.^{31,32} A discrete source for disseminated infection often is unclear whether the disease is due to primary infection or reactivation. The clinical syndrome of PDH consists of respiratory symptoms with prolonged fever,

fatigue, weight loss, and night sweats. In patients with disseminated histoplasmosis, an enlarged liver and spleen may be found. In addition, diffuse lymphadenopathy is often found.^{23,24,27} The spectrum of this PDH in HIV-infected patients is not specific and hard to distinguish from other infectious diseases, such as disseminated TB, therefore delay the diagnosis.^{33–35} Laboratory of a patient with PDH often reveals pancytopenia, elevated liver enzymes, elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), and ferritin.^{23,32,36–38} The most common dissemination PDH is the reticuloendothelial system, including liver, spleen, bone marrow, and lymph nodes. The oral mucosa also has been reported as the most affected site of dissemination.^{23,27} Meanwhile, the skin is not a common extrapulmonary site of PDH, except in patients with advanced HIV infection.^{23,39} The cutaneous involvement lesions vary from papules, pustules, plaques, ulcers, wart-like lesions, and even erythema nodosum.³⁹ These lesions are usually affected in the face, extremities, and trunk.⁴⁰ A nonspecific clinical features of PDH cause history of patients living in endemic areas or probably has recent epidemiologic exposures should be maintained in immunocompromised patients.^{23,27}

LABORATORY DIAGNOSTIC METHODS

The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) have defined criteria for a proven diagnosis of invasive fungal from dimorphic fungi. A proven (gold standard) criteria for histoplasmosis diagnosis is a positive culture or histopathology or direct microscopy of Hc from clinical specimens (bone marrow, blood, biopsied tissue, sterile fluids, or respiratory specimens). If all proven criteria are not available or showed a negative result, but the patient is immunocompromised with typical clinical features, evidence of environmental exposure to the fungus, and the presence of *Histoplasma* antigen in any body fluid, the diagnosis is considered probable.⁴¹ Laboratory methods

for diagnosis histoplasmosis include direct examination, culture, histopathology, antigen/antibody detection, and molecular. These tests' sensitivity is related to several variables, consisting of the patient's clinical form (acute/chronic pulmonary or disseminated), immune status, clinical specimens, and fungal burden.⁴²

Direct examination

Direct examination of clinical specimens consists of KOH wet slide examination and histopathology investigation.

KOH wet slide

Examination under microscope using potassium hydroxide (KOH 10%) is a rapid way still have very low sensitivity and specificity for diagnosis.^{43,44} Meanwhile, calcofluor white stain examination under the fluorescent microscope may increase Hc's detection; however, the yeast visualization is not pathognomonic.⁴² Furthermore, the direct examination requires highly trained professionals to make a proper diagnosis.

Histopathology

Histopathologic examination using specific stains that exhibit Hc's yeast-like structures is a definitive and rapid way for histoplasmosis diagnosis, especially disseminated form. The

Table 1. The sensitivity of laboratory methods based on the patient's clinical form of histoplasmosis^{49,50,58}

Methods	Acute pulmonary	Chronic pulmonary	Progressive disseminated
Culture	0 (3), 42 (19)*	66.7 (6)^	74.2 (132)^
Pathology	0 (2), 20 (10)*	75 (4)^	76.3 (76)^
Antigen detection	83.3 (6), 83 (29)*	87.5 (8)^	91.8 (158)^
Antibody detection	66.7 (6), 64 (28)*	83.3 9 (6)^	75 (80)^
Molecular	NA	NA	95 (NA)#

All data showed in percentage (%), The number in parentheses represents the number of patients who were tested ^ cited from:[49], *cited from:[50], #cited from: [58]

samples could be obtained from tissues or body fluids.^{41,42,44} The sample-taking procedure of touch biopsy is less invasive and feasible for diagnosis of disseminated histoplasmosis with cutaneous involvement.⁴⁵ However, a clinical specimen from other sites such as bone marrow aspiration and biopsy is invasive. Furthermore, professional expertise

is mandatory to differentiate Hc yeast from other fungal pathogens such as *Blastomyces dermatitis*, *Candida glabrata*, *Coccidioides* sp, *Cryptococcus* sp, *Emergomyces* sp, *Pneumocystis jirovecii*, *Talaromyces marneffeii*, and *Leishmania donovani*.^{18,46,47} The sensitivity of the pathology assay varies according to histoplasmosis's clinical manifestation (Table 1).

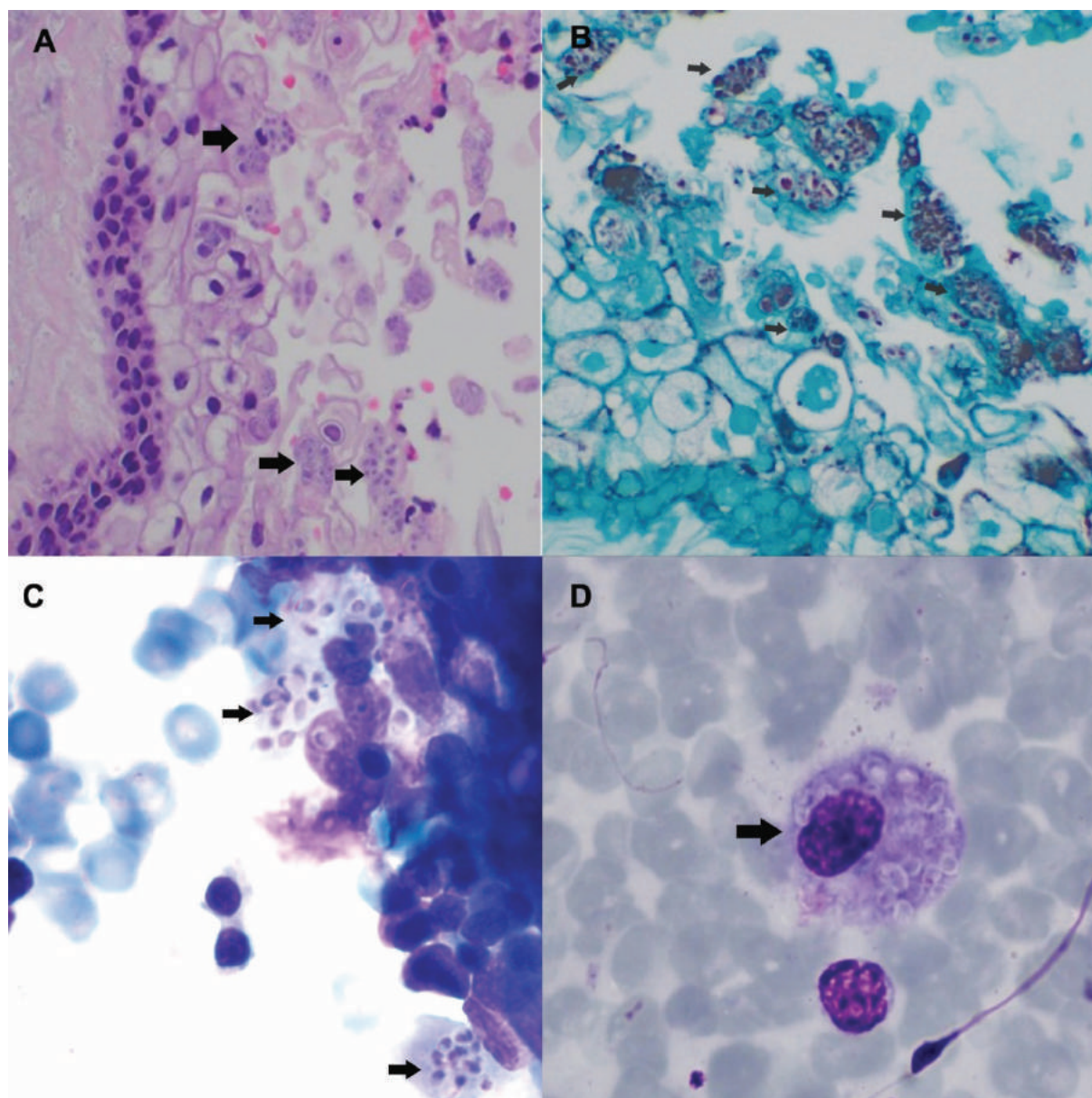


Figure 1. Histopathologic examination of *Histoplasma* sp. A) Umbilical cord with HE staining, B) Umbilical cord with GMS, C) Bone marrow aspirate smear with Wright - Giemsa, D) Touch biopsy of the skin with Giemsa. The arrows are demonstrating numerous ovoid intracellular yeast cells surrounded by hyaline halo within macrophage (1000 × magnification). Images A and B courtesy of Dr. Kenneth Chang and Dr. Aw Sze Jet, Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital; Singapore. Images C and D courtesy of R. Wahyuningsih, Parasitology Laboratory, Faculty of Medicine, Universitas Indonesia.

Histoplasma capsulatum characteristic yeasts are ovoid with a clear halo-like capsule surrounding, divide by narrow-based budding, and predominantly intracellular (within macrophages and giant cells). The yeast diameter measure is around 2-4 μm .³⁵ Specific pathologic stains for identifying yeast cells of Hc include hematoxylin and eosin (H&E), Gomori methenamine-silver (GMS), or periodic acid-Schiff (PAS). Meanwhile, Wright-Giemsa stains may be used to rapidly discover yeast cells of Hc in peripheral blood or bone marrow aspirate smears (Figure 1).^{46,48}

Culture

Histoplasma isolation in culture is still considered the gold standard for diagnosing histoplasmosis. Nevertheless, the process is so time-consuming and requires a biosafety level 3 laboratory. Clinical specimens from patients suspected of histoplasmosis might be inoculated onto an appropriate culture medium, e.g., Sabouraud dextrose agar (SDA) or Brain-Heart infusion (BHI) agar. When culture incubated at 25-30°C, it needs 4 to 6 weeks to be detectable as a mold with cream to brown cottony colonies (Figure 2A). After a colony is

identified on medium, a lactophenol cotton blue or lactoglycerol/ lactophenol slide can be performed to establish mold morphology. Microscopic identification of mold morphology will show septated hyphae at first, followed by microconidia based on the maturity of the mycelia phase. The smooth microconidia diameter varies from 2 to 5 μm , followed by tuberculate macroconidia form with 7 to 15 μm in diameter (Figure 2B).^{24,42}

Since Hc is dimorphic fungi, if the mold form incubates at 35-37°C, it will transform into yeast phase and commonly occurs within 2 to 4 weeks. Previously conversion method was used as a confirmed diagnosis, but the conversion rate is low, so developed countries have left it behind.^{25,42} The sensitivity of the culture-based methods is dependent on the fungal burden. Samples from patients with disseminated histoplasmosis reveal higher sensitivity which is 74%, than in acute pulmonary histoplasmosis is 42% (Table 1).^{49,50}

Antigen detection

Antigen detection is a laboratory method that allows a less invasive, rapid, and sensitive diagnosis of acute and disseminated histoplasmosis

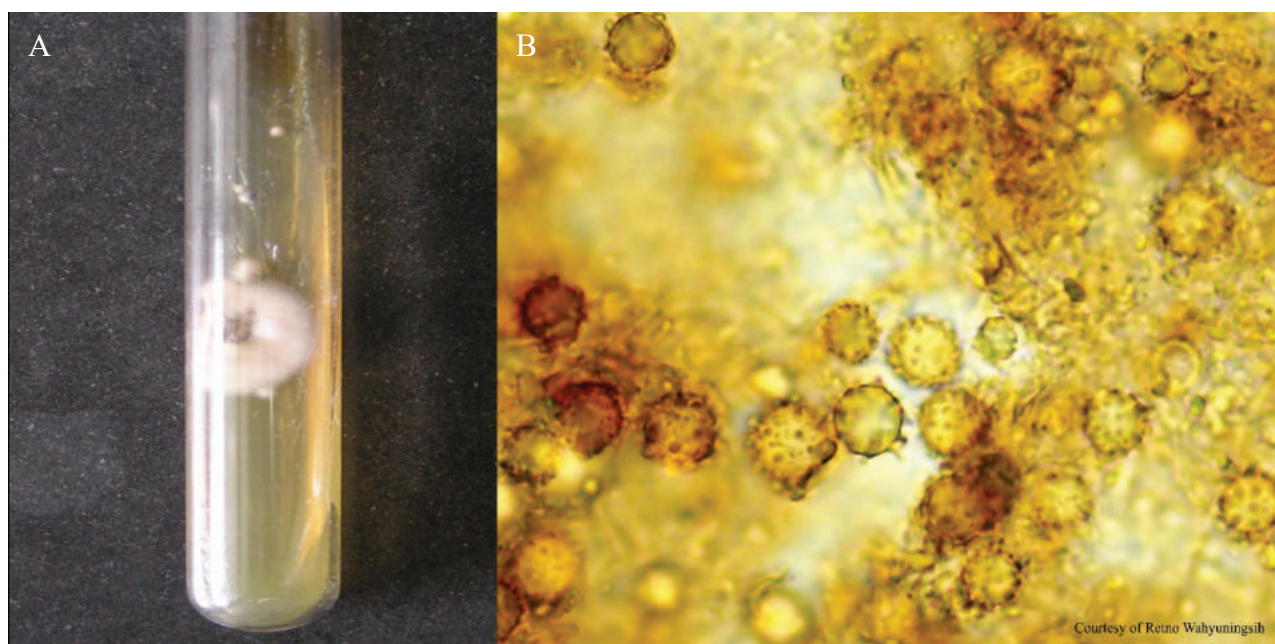


Figure 2. A) Culture of *Histoplasma capsulatum* at 25°C, B) Microscopic of lactophenol/lactoglycerol stained showed tuberculate macroconidia, a characteristic morphological structure of *Histoplasma* spore, 400 \times magnification. (Images courtesy of R. Wahyuningsih (Parasitology laboratory, Faculty of Medicine, Universitas Indonesia).

where the burden of infection is high.¹⁸ During infection with Hc, antigens

are released by fungal cells into the tissues and enter body fluids adjacent to the site of infection, such as serum (blood) fluids, bronchoalveolar lavage (BAL), urine, and cerebrospinal fluid (CSF). It allows as a useful method for rapid diagnosis of histoplasmosis and for monitoring the effect of therapy.^{17,18,48,51}

Antigen assay for histoplasmosis was first developed in 1986 using a sandwich radioimmunoassay in urine and serum specimens—a radioimmunoassay assay based on detection of polyclonal rabbit antibodies against *Histoplasma* galactomannan. In 1997, the *Histoplasma* antigen detection method was adapted to enzyme immunoassay (EIA). The EIA method was then developed into the second generation in 2004 to prevent false-positive results due to human anti-rabbit antibodies. In 2007, the third generation of *Histoplasma* EIA examination was launched with a quantification test's superiority with higher specificity (MiraVista *H. capsulatum* Galactomannan EIA). This EIA examination can be done with a kit from MiraVista Diagnostics. However, the test can only be done in-house compared to the ALPHA *Histoplasma* EIA test from IMMY commercially available and does not have to be in its laboratory. The use of the kit from IMMY then also received approval from the Food and Drug Administration (FDA) on urine specimens and can be used in local facilities. Besides, the IMMY test was recently modified using monoclonal antibodies, which significantly increased its sensitivity.⁴²

Histoplasma antigen tests on urine specimens generally proved slightly more sensitive than serum for diagnosing all forms of histoplasmosis. Moreover, combining both urine and serum testing enhances the sensitivity of antigen detection.²⁵ In a recent study, specimens from BAL fluid may increase histoplasmosis diagnosis sensitivity.⁵² Other specimens such as CSF may be useful too in the diagnosis of *Histoplasma* meningitis.⁵³ A meta-analysis study in 2016 that evaluated the diagnostic performance of the *Histoplasma* antigen test showed that the overall sensitivity of the test in serum and urine was 81% while the

specificity was 99%.⁵⁴ While the sensitivity based on clinical manifestations varied (Table 1).

A limitation from the *Histoplasma* antigen assay is the remarkable cross-reactivity with other dimorphic fungal, such as *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Talaromyces marneffeii*, *Coccidioides immitis*, and *Emergomycetes* sp. Another cross-reactivity has also been reported with Platelia *Aspergillus* EIA which the false-positive result is correlated with the concentration of positivity *Histoplasma* antigen EIA.⁵⁵ Unfortunately, the antigen detection kit for histoplasmosis not yet available in Indonesia. Thus, meticulously morphological identification is the primary key for laboratory clinicians.

Antibody detection

Antibody detection-based tests may provide a rapid diagnosis; however, unsuitable for an early acute form of infection Hc. The newly formed antibodies can be detected in peripheral blood about 4 to 8 weeks after exposure to Hc and then persist for years.^{26,42} Therefore, antibody detection is more suitable for diagnosing subacute and chronic forms of histoplasmosis (Table 1). Besides, this test has significant limitations, particularly in immunocompromised patients, who are unable to enhance the humoral immune response, so false negatives can occur.⁵⁶

The available methods of antibody detection tests against Hc are EIA, complement fixation (CF), immunodiffusion (ID), latex agglutination (LA), and Western blot (WB).^{42,44} The most sensitive antibody detection test for histoplasmosis is EIA but has high false-positives results. The low specificity of the EIA test makes it has not been standardized across laboratories.²⁶ The CF and ID are generally performed in reference laboratories due to their reliability and potential cost-effectiveness.⁴⁸ The CF performance is more sensitive than ID but less specific. The ID detects antibodies that bind to H and M fungal antigens of Hc. The H band (20%) is rarely seen as M band (80%). However, when H band is present indicates acute infection. Meanwhile, the M band is more commonly seen but couldn't tell the difference between active from latent infection or resolved histoplasmosis.⁴² The ID specificity is 100%, whereas sensitivity ranges from 70 to

95%, depending on histoplasmosis's form.⁴⁸ The commercial LA tests, which are commercially available, showed false positives in patients with TB and should be aware by clinicians.¹⁸ Currently, a study to validate the WB assay for diagnosis histoplasmosis showed high sensitivity (94.9%) and specificity (94.1%).⁵⁷

Molecular

Molecular methods that can detect *Histoplasma* DNA for diagnosis are highly specific. Nonetheless, there are no commercial and FDA-approved molecular tests to detect Hc from clinical specimens directly.⁴⁴ DNA probes exist commercially but are used for definite identification from positive cultures rather than from direct clinical specimens. Thus, several studies are currently developing direct

Hc detection trials from clinical samples.⁴² The performance of the molecular tests in the diagnosis varies according to the clinical form of histoplasmosis. A meta-analysis study showed the overall sensitivity of the molecular tests from different types of specimens, e.g., tissue biopsy, respiratory, blood, and bone marrow samples for diagnosing disseminated histoplasmosis was 95%, and the specificity was 99% (Table 1).⁵⁸

TREATMENT

The recommended therapy for histoplasmosis differs according to the patient's clinical spectrum (Table 2). Treatment is indicated in patients with severe or moderately severe acute pulmonary, chronic cavitary pulmonary, disseminated, and central nervous system (CNS) histoplasmosis. The

Table 2. Treatment of histoplasmosis according to the patient's clinical spectrum^{44,59,60}

Clinical spectrum of histoplasmosis	Recommendation	Notes
Acute pulmonary (moderately severe or severe)	AmB lipid complex (3-5 mg/kg/day IV) or d-AmB (0.7-1 mg/kg/day IV) for 1-2 weeks or until the patient is clinically stable, then switch to itraconazole 200 mg PO two times a day for ≥ 12 months	One liter of normal saline (NaCl 0.9%) can be administered before administration of d-AmB to reduce the nephrotoxicity effect. Most available in Indonesia is d-Amb
	Methylprednisolone (0.5–1 mg/kg/day IV) for 1–2 weeks	Combination with steroid is recommended for patients who develop respiratory complications, e.g., hypoxemia/ significant respiratory distress
Acute pulmonary (mild to moderate but with symptoms > 4 weeks)	Itraconazole PO 200 mg two times a day for 6-12 weeks	Absorption of the capsule formulation is affected by gastric pH, which is improved if given with food and carbonated drink
Chronic cavitary pulmonary	Itraconazole PO 200 mg two times a day 12 months	Treatment could be extended to 18–24 months to minimize the risk for relapse
Progressive disseminated	Liposomal AmB (3 mg/kg/day IV) or AmB lipid complex (3-5 mg/kg/day IV) or AmB deoxycholate (0.7-1 mg/kg/day IV) for 1-2 weeks or until the patient is clinically stable then switch to itraconazole PO 200 mg 3 times a day for three days, and then 200 mg two times a day for ≥ 12 months.	More prolonged treatment may be required in patients with persistent immunosuppression condition
CNS histoplasmosis	Liposomal AmB (5 mg/kg/day IV) for 4–6 weeks, then switch to itraconazole 200 mg PO two/ three times a day for ≥ 12 months	

Description: AmB: amphotericin B; d-AmB: Amphotericin B deoxycholate; IV: intravenous; PO: peroral

antifungal agent that has been proven to be effective include amphotericin B (liposomal amphotericin B, amphotericin B lipid complex, amphotericin B deoxycholate) and itraconazole.⁵⁹

Amphotericin B deoxycholate (d-AmB) is a polyene antifungal agent administered intravenously by infusion in 2-4 hour intervals. The dosage for histoplasmosis is 0.7-1 mg/kg/day and can be given approximately two weeks. The administration of this drug may cause adverse side effects, divided into acute and chronic reactions. Feasible acute reactions include chills, fever, and tachypnea, which occur 30 to 45 minutes after the first dose. Premedication with paracetamol or corticosteroids can be given to diminish the reaction. Its chronic adverse effect is nephrotoxicity, which is dose and infusion-related. The mechanism of nephrotoxicity due to d-AmB is decreasing glomerulotubular renal flow. Isotonic saline administration before d-AmB may preserve renal function. Clinical features of other kidney effect include hypokalemia, and distal renal tubular acidosis, which can be treated with potassium and bicarbonate.⁶⁰

The primary azole group for histoplasmosis treatment is itraconazole that can be given for 6-9 months. Fluconazole may be utilized as a second-line agent but not as efficacious as itraconazole. Other azole antifungal agents such as voriconazole and posaconazole may be effective for histoplasmosis treatment; however, there are inadequate clinical experience data. While the echinocandins should not be used because they showed limited in vitro activity against Hc.^{27,44,59}

SUMMARY

Histoplasmosis is a disease caused by the fungus Hcc, which is distributed worldwide. The infection occurred when the conidia of Hc inhaled and transformed into yeasts in the lungs. This fungal infection can cause symptoms in immunocompromised and immunocompetent patients. The majority of patients are self-

limited mild pulmonary infections remarkably never recognized as being histoplasmosis in immunocompetent individuals. However, in immunocompromised patients or extreme of age or following inhalation, a large inoculum typically presents as a pulmonary disease with diverse manifestations. The

Clinical features include acute pulmonary histoplasmosis, chronic cavitary pulmonary histoplasmosis, a complication of pulmonary histoplasmosis, and progressive disseminated histoplasmosis (PDH).

Isolation of Hc from clinical specimens remains the gold standard for the diagnosis of histoplasmosis. However, it takes 4-6 weeks to be identified and needs a high laboratory requirement. Alternately histopathology methods with Wright-Giemsa stains may be used to rapidly detect yeast cells of Hc in peripheral blood or bone marrow aspirate smears.

In recent years, the number of immunocompromised individuals and travelers from and to endemic areas histoplasmosis increases. Also, Indonesia has HST sensitivity above the global scale of histoplasmin sensitivity, which supports an endemic area, e.g., Jakarta, Surabaya, Bali, and Medan in the 1900s. Therefore, physicians need to be aware of this infection because the clinical feature can mimic other pulmonary diseases. A careful history of possible exposure and the appropriate diagnostic approach described above is essential to provide appropriate therapy.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Darling ST. A protozoön general infection producing pseudotubercles in the lungs and focal necroses in the liver, spleen and lymphnodes. J Am Med Assoc. Epub ahead of print 1906. DOI: 10.1001/jama.1906.62510440037003.
2. Schwarz J, Baum GL. The history of histoplasmosis, 1906 to 1956. N Engl J Med. 1957;256: 253–258.
3. Bahr NC, Antinori S, Wheat LJ, et al. Histoplasmosis infections worldwide: thinking outside of the Ohio River valley. Curr Trop Med reports. 2015;2:70–80.
4. Kasuga T, White TJ, Koenig G, et al. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. Mol Ecol. 2003;12:3383–401.
5. Teixeira M de M, Patané JSL, Taylor ML, Gómez BL, Theodoro RC, de Hoog S, et al. Worldwide Phylogenetic Distributions and Population Dynamics of the Genus *Histoplasma*. PLoS Negl Trop Dis. 2016;10(6):e0004732.
6. Dubois A, Janssens PG, Brutsaert P, Vanbreuseghem R. A case of African histoplasmosis; with a mycological note on *Histoplasma duboisii* n.sp. Ann la Soc belge Med Trop. 1952;32(6):569–84.
7. Edwards JA, Rappleye CA. *Histoplasma* mechanisms of pathogenesis - one portfolio does not fit all. FEMS Microbiology Letters. 2011;324:1–9.
8. Mittal J, Ponce MG, Gendlina I, et al. *Histoplasma capsulatum*: Mechanisms for pathogenesis. In: Curr Top Microbiol Immunol. 2019;422:157-191.
9. Deepe GS. Outbreaks of histoplasmosis: The spores set sail. Sheppard DC, editor. PLOS Pathog. 2018;14(9):e1007213.
10. Mihi MR, Nosanchuk JD. *Histoplasma* virulence and host responses. Int J Microbiol. 2012;2012:268123.
11. Randhawa HS, Gugnan HC. Occurrence of Histoplasmosis in the Indian Sub-Continent: An Overview and Update. J Med Res Pr. 2018;07: 71–83.
12. Boigues BCS, Paniago AMM, Lima GME, et al. Clinical outcomes and risk factors for death from disseminated histoplasmosis in patients with AIDS who visited a high-complexity hospital in campo Grande, MS, Brazil. Rev Soc Bras Med Trop. 2018;51:155–161.
13. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR Suppl 1987;36(1):1S-15S
14. Chander J. Histoplasmosis. In: Chander J, ed. Textbook of Medical Mycology. 4th ed. New Delhi, India: Jaypee Brothers Medical Publishers Ltd; 2018:311–23.
15. Benedict K, Mody RK. Epidemiology of histoplasmosis outbreaks, United States, 1938-2013. Emerg Infect Dis. 2016;22(3):370–8.
16. Müller H. Histoplasmosis in East Java. Geneeskd Tijdschr voor Ned. 1932;72(14).
17. Wheat LJ. Current diagnosis of histoplasmosis. Trends Microbiol. 2003;11(10):488–94.
18. Guimarães AJ, Nosanchuk JD, Zancopé-Oliveira RM. Diagnosis of Histoplasmosis. Braz J Microbiol. 2006;37(1):1-13
19. Baker J, Setianingrum F, Wahyuningsih R, Denning DW. Mapping histoplasmosis in South East Asia – implications for diagnosis in AIDS. Emerg Microbes Infect. 2019;8(1):1139–45.
20. Anggorowati N, Sulistyarningsih RC, Ghozali A, Subronto YW. Disseminated Histoplasmosis in an Indonesian HIV-Positive Patient: A Case Diagnosed by Fine Needle Aspiration Cytology. Acta Med Indones. 2018 Jan 19;49(4):360.
21. Hartono A, Soeprihatin S. Dua kasus dengan kelainan pada pharynx jang djarang ditemukan. Oto Rhino Laryngol Indon. 1970;2:17–32.
22. Abdulsalam M, Hoedijoko, Gatot D, et al. Histoplasmosis diseminata pada anak. Medika. 1986;12:4–9.
23. Linder KA, Kauffman CA. Histoplasmosis: Epidemiology, Diagnosis, and Clinical Manifestations. Current Fungal Infection Reports. 2019;13(3):120-8.
24. Kauffman CA. Histoplasmosis: A clinical and laboratory update. Clinical Microbiology Reviews. American Society for Microbiology (ASM); 2007;20(1):115-32.
25. Hage CA, Azar MM, Bahr N, Loyd J, Wheat LJ. Histoplasmosis: Up-to-Date Evidence-Based Approach to Diagnosis and Management. Semin Respir Crit Care Med. 2015; 36(5):729-45.
26. Azar MM, Hage CA. Clinical Perspectives in the Diagnosis and Management of Histoplasmosis. Clinics in Chest Medicine. W.B. Saunders; 2017;38(3):403-15.
27. Azar MM, Loyd JL, Relich RF, Wheat LJ, Hage CA. Current Concepts in the Epidemiology, Diagnosis, and Management of Histoplasmosis Syndromes. Semin Respir Crit Care Med. 2020;41(1):13–30.
28. Staffolani S, Buonfrate D, Angheben A, et al. Acute histoplasmosis in immunocompetent travelers: A systematic review of literature. BMC Infectious Diseases. 2018;18:673.
29. Baker J, Kosmidis C, Rozaliyani A, Wahyuningsih R, Denning DW. Chronic Pulmonary Histoplasmosis-A Scoping Literature Review. Open Forum Infect Dis. 2020;7(5):ofaa119.
30. Kandi V, Vaish R, Palange P, Bhoomagiri MR. Chronic Pulmonary Histoplasmosis and its Clinical Significance: an Under-reported Systemic Fungal Disease. Cureus. 2016;8(8):e751.
31. Samaddar A, Sharma A, Kumar PHA, et al. Disseminated histoplasmosis in immunocompetent patients from an arid zone in Western India: A case series. Med Mycol Case Rep. 2019;25:49–52.
32. Xiong XF, Fan LL, Kang M, Wei J, Cheng DY. Disseminated histoplasmosis: A rare clinical phenotype

- with difficult diagnosis. *Respirol Case Reports*. 2017;5(3):e002.
33. Jeong HW, Sohn JW, Kim MJ, Choi JW, Kim CH, Choi SH, et al. Disseminated histoplasmosis and tuberculosis in a patient with HIV infection. *Yonsei medical journal*. 2007;48(3):531-4.
 34. Nacher M, Couppié P, Epelboin L, Djossou F, Demar M, Adenis A. Disseminated Histoplasmosis: Fighting a neglected killer of patients with advanced HIV disease in Latin America. *PLoS pathogens*. 2020 May 14;16(5):e1008449.
 35. Murray M, Hine P, Garner P. Guidelines for Diagnosing and Managing Disseminated Histoplasmosis among People Living with HIV [Internet]. PAHO;2020. [cited 25 January 2021]. Available from: <https://iris.paho.org/handle/10665.2/5230>.
 36. Kutkut I, Vater L, Goldman M, Czader M, Swenberg J, Fulkerson Z, et al. Thrombocytopenia and disseminated histoplasmosis in immunocompetent adults. *Clin Case Reports*. 2017;5(12):1954–60.
 37. Choi J, Nikoomanesh K, Uppal J, Wang S. Progressive disseminated histoplasmosis with concomitant disseminated nontuberculous mycobacterial infection in a patient with AIDS from a non-endemic region (California). *BMC Pulm Med*. 2019;19(1):46.
 38. Zarlisht F, Zarlisht F, Ramadan M, Almoadhen M, Lin K, Khaja M, et al. Lactate Dehydrogenase and Ferritin Levels: A Clinical Clue for Early Diagnosis of Disseminated Histoplasmosis in HIV Patients. *J Med Cases*. 2016;7(3):81–3.
 39. Smith JA, Riddell J, Kauffman CA. Cutaneous manifestations of endemic mycoses. *Current Infectious Disease Reports*. 2013;15: 440–449.
 40. Chang P, Rodas C. Skin lesions in histoplasmosis. *Clinics in dermatology*. 2012;30(6):592-8.
 41. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clinical Infectious Diseases*. 2019.
 42. Azar MM, Hage CA. Laboratory diagnostics for histoplasmosis. *Journal of Clinical Microbiology*. 2017;55(6):1612-20.
 43. Nacher M, Blanchet D, Bongomin F, Chakrabarti A, Couppié P, Demar M, et al. *Histoplasma capsulatum* antigen detection tests as an essential diagnostic tool for patients with advanced HIV disease in low and middle income countries: A systematic review of diagnostic accuracy studies. *PLoS Negl Trop Dis*. 2018;12(10):e0006802.
 44. Almeida-Silva F, Gonçalves D de S, de Abreu Almeida M, Guimarães AJ. Current Aspects of Diagnosis and Therapeutics of Histoplasmosis and Future Trends: Moving onto a New Immune (Diagnosis and Therapeutic) Era?. *Current Clinical Microbiology Reports*. 2019;15;6(3):98-107.
 45. Wahyuningsih R, Adawiyah R, Suriadiredja A, Sjam R, Yuniastuti E, Imran D, et al. Touch Biopsy: A Simple and Rapid Method for the Diagnosis of Systemic Mycoses with Skin Dissemination in HIV-Infected Patients. *International Journal of Technology*. In Press 2021.
 46. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clinical Microbiology Reviews*. 2011;24(2);247-80.
 47. Schwartz IS, Govender NP, Sigler L, Jiang Y, Maphanga TG, Toplis B, et al. *Emergomycetes*: The global rise of new dimorphic fungal pathogens. Hogan DA, editor. *PLOS Pathog*. 2019;15(9):e1007977.
 48. Scheel CM, Gómez BL. Diagnostic Methods for Histoplasmosis: Focus on Endemic Countries with Variable Infrastructure Levels. *Current Tropical Medicine Reports*. 2014;1(2):129-37.
 49. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis*. 2011;53(5):448-54.
 50. Swartzentruber S, Rhodes L, Kurkjian K, Zahn M, Brandt ME, Connolly P, et al. Diagnosis of Acute Pulmonary Histoplasmosis by Antigen Detection. *Clin Infect Dis*. 2009;49(12):1878–82.
 51. Wheat LJ, Garringer T, Brizendine E, et al. Diagnosis of histoplasmosis by antigen detection based upon experience at the histoplasmosis reference laboratory. *Diagn Microbiol Infect Dis*. 2002;43: 29–37.
 52. Hage CA, Davis TE, Fuller D, Egan L, Witt JR, Wheat LJ, et al. Diagnosis of histoplasmosis by antigen detection in BAL fluid. *Chest*. 2010;137(3):623–8.
 53. Bloch KC, Myint T, Raymond-Guillen L, Hage CA, Davis TE, Wright PW, et al. Improvement in Diagnosis of *Histoplasma* Meningitis by Combined Testing for *Histoplasma* Antigen and Immunoglobulin G and Immunoglobulin M Anti-*Histoplasma* Antibody in Cerebrospinal Fluid. *Clin Infect Dis*. 2018;66(1):89–94.
 54. Fandiño-Devia E, Rodríguez-Echeverri C, Cardona-Arias J, Gonzalez A. Antigen Detection in the Diagnosis of Histoplasmosis: A Meta-analysis of Diagnostic Performance. *Mycopathologia*. 2016;181(3–4):197–205.
 55. Wheat LJ, Hackett E, Durkin M, Connolly P, Petraitiene R, Walsh TJ, et al. Histoplasmosis-associated cross-reactivity in the Biorad Platelia *Aspergillus* enzyme immunoassay. *Clin Vaccine Immunol*. 2007;14(5):638–40.
 56. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. Histoplasmosis. *Infect Dis Clin North Am*. 2016;30(1):207–27.
 57. Almeida M de A, Pizzini CV, Damasceno LS, Muniz M de M, Almeida-Paes R, Peralta RHS, et al. Validation

- of western blot for *Histoplasma capsulatum* antibody detection assay. *BMC Infect Dis.* 2016;16(1):87.
58. Caceres DH, Knuth M, Derado G, Lindsley MD. Diagnosis of progressive disseminated histoplasmosis in advanced HIV: A meta-analysis of assay analytical performance. *J Fungi.* 2019;5(3):76.
59. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, et al. Clinical Practice Guidelines for the Management of Patients with Histoplasmosis: 2007 Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2007;45(7):807–25.
60. Stevens DA. Systemic antifungal agents. In: Goldman's Cecil Medicine. WB Saunders; 2012:1971–77.

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Research Article

Manifestations of Acute Pancreatitis in Severe COVID-19 Patients: Is This a Coincidence?

Pradana Zaky Romadhon¹, Satriyo Dwi Suryantoro^{2*}, Choirina Windradi², Bagus Aulia Mahdi¹, Esthiningrum Dewi Agustin², Krisnina Nurul W¹, Dwiki Novendrianto¹

¹Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Universitas Airlangga Hospital, Surabaya, Indonesia

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ABSTRACT

Coronavirus Disease-19 (COVID-19) adalah penyakit yang disebabkan oleh Severe Acute Respiratory Coronavirus-2 (SARS-CoV2) yang berasal dari China, menyebar dengan cepat ke seluruh bagian negara lain yang menyebabkan pandemi dunia. Dengan derajat gejala yang bervariasi yang disebabkan oleh COVID-19, virus ini menyebabkan kerusakan pada beberapa organ, baik karena efek inflamasi tidak langsung maupun efek sitopatik. Data terkait keterlibatan pankreas dalam kasus COVID-19 masih belum jelas. Seorang laki-laki usia 83 tahun dirawat karena gejala COVID-19 berat. Dalam perawatan, pasien memberikan gejala dan tanda pankreatitis akut tanpa diketahui faktor risiko yang terkait. Pada pemeriksaan didapatkan RT-PCR SARS-CoV2 positif dari swab nasofaring, amilase lipase yang meningkat serta gambaran ultrasound khas untuk pankreatitis akut. Tatalaksana pasien tetap berdasar pada kasus SARS-CoV2 dengan isolasi, oksigenasi, pemberian anti virus dan suportif. Pemberian antibiotik juga didasarkan pada terapi empiris yang kemudian disesuaikan hasil sensitivitas kultur. Skor prognosis pankreatitis menunjukkan risiko kematian pada kasus moderate. Pada perjalanan, pasien meninggal karena shock sepsis. Prevalensi pankreatitis akut dan tingkat keparahannya perlu diamati. Dalam artikel ini, kami menyajikan kasus pankreatitis akut yang terjadi pada COVID-19 parah dengan faktor risiko yang tidak diketahui. Diagnosis penyebab kasus pankreatitis masih belum jelas tetapi beberapa bukti autopsi kasus infeksi SARS-CoV2 dengan pankreatitis menyebutkan bahwa infeksi virus ini dapat menyebabkan injuri pada pankreas. Kondisi sepsis dapat diakibatkan infeksi virus SARS-CoV2 (viral sepsis) atau ko-infeksi bakteri. Oleh karena itu, rasionalisasi penggunaan antibiotik juga diperlukan. Kasus ini merupakan kasus yang membutuhkan manajemen holistik dan intensif karena kedua kondisi berpotensi dapat memperberat satu sama lain. Pengenalan awal kegawatan serta terapi tepat merupakan hal yang penting dapat menunjang kesintasan pasien.

Keywords: Pancreatitis, Infectious disease, COVID-19, Organ damage, Sepsis

ABSTRACT

Coronavirus Disease-19 (COVID-19) is a disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV2) came from China, this disease is highly infectious causing rapid spread throughout the world. COVID-19 had various of symptoms, manifestation, and also degree to cause multiorgan dysfunctions either due to indirect inflammatory effects or cytopathic effects. Data regarding the involvement of the pancreas in COVID-19 cases is still unclear. An 83-year-old man was being treated for severe COVID-19 symptoms. He had received treatment for severe COVID-19. Unfortunately, during hospitalization, the patient presented the symptoms and signs of acute pancreatitis without any known risk factors. Physical findings supported the diagnosis criteria for acute pancreatitis. Moreover, supporting examination found a positive SARS-CoV2 RT-PCR from a nasopharyngeal swab, increased amylase lipase and a typical ultrasound image for acute pancreatitis. Patient management was remains based on COVID-19 cases with consisting of isolation, oxygenation, antiviral and other supportive medical treatment. Antibiotic administration was also based on empirical therapy which was then adjusted for the results of culture sensitivity. Although the etiology diagnosis of this patient was uncertain, we assumed SARS-CoV2 infection could cause injury to the pancreas.

* Corresponding Author:
satriyo.dwi.suryantoro@fk.unair.ac.id

We did observe patient based on clinical and laboratory findings other than that based on Ranson's Score the patient was in poor prognosis. Eventually patient died due to septic shock. Sepsis conditions in COVID-19 patients could be due to viral sepsis and bacterial co-infection. Therefore, a rationalization of the use of antibiotics is also needed. This case is a case that requires intensive and holistic management because the two conditions can potentially aggravate each other. Early recognition of emergency and appropriate therapy is important to support patient survival.

Kata kunci: pankreatitis, pandemi, COVID-19, kerusakan organ, sepsis

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INTRODUCTION

SARS-CoV2 causes COVID-19 infection that until now has become a world pandemic¹⁻³. Coronavirus (CoV) is a group of enveloped viruses possibly identified in animals. Some CoV found in animals may precipitate infectious diseases, such as viral gastroenteritis (TGEV), porcine epidemic diarrhea virus (PEDV), avian infectious bronchitis virus (IBV), and swine acute diarrhea syndrome coronavirus (SADS-CoV)⁴⁻⁶. COVID-19 also came with extrapulmonary manifestations which involve the role of the ACE2 receptor. This may include neurological, renal, hepatic, gastrointestinal, thromboembolic, cardiac, endocrine, and dermatology systems^{7, 8}. To date, the most common gastrointestinal manifestations of COVID-19 are nausea, vomiting, diarrhea, abdominal pain⁹⁻¹⁵. However, in our case, the occurrence of acute pancreatitis without an understandable risk factor was the direct injury to the pancreas gland by SARS-CoV2¹⁶.

CASE

Mr. S, 83 years old, came with tightness 2-3 days ago getting more severe for two days. The patient did not previously complain of having fever and runny nose. There are no complaints of nausea or vomiting. Sometimes the patient complains of abdominal pain so that he is merely able to eat half of the usual portion. Daily, the patient can still do activities such as bathing, walking, and wearing clothes. However, since

then, the patient was unable to carry out his activity. He was weak and only able to lie down and sit with assistance. The patient started having a cough 2 days ago, thus the abdominal tightness was getting worse when coughing. The patient self-checks peripheral oxygen saturation at home and turned out only 72-75% room air. The patient did not previously have diabetes mellitus but had a history of hypertension with heart disease. There is no history of drinking alcohol. The patient takes Clopidogrel 75 mg once daily and Bisoprolol 2.5 mg once daily.

Respiratory muscles were found retracted. Neither Ronchi nor wheezing was heard from the lung examination. The apex beat is dilated, which is in line with cardiomegaly figured out on x-ray. Initial examination revealed a positive SARS-CoV2 antigen swab with a chest x-ray showing pneumonia (see Figure 1). Naso-oropharynx polymerase chain reaction (PCR) swab was positive for SARS-CoV2 with CT 27.5.

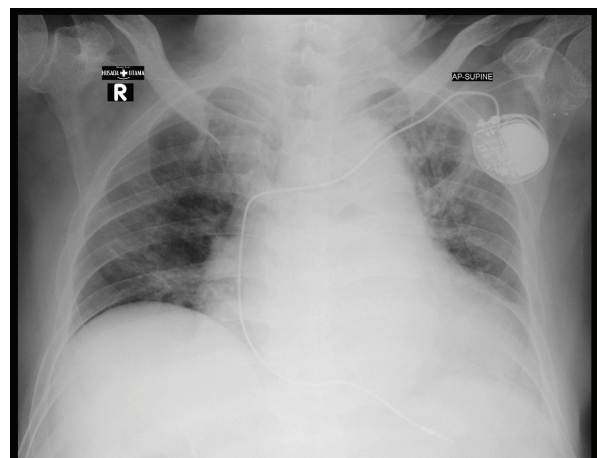


Figure 1. Pneumonia COVID-19 chest x-ray.

Laboratory findings showed Hb 14.2 g/dl, white blood count 6530 u/l, urea 69.8; serum creatinine 2.84, D-dimer 8.54. Blood gas analysis showed moderate to severe hypoxia with pH 7.43; pCO₂ 24, pO₂ 146, BE 17, HCO₃ 16.2, SaO₂ 96. The patient received supportive and symptomatic treatments such as oxygen supplementation, Meropenem, Nebivolol, Codeine, Dexamethasone for ten days, Fondaparinux, and Remdesivir drip for five days.

On the third day of treatment, we found that the patient's right upper abdominal pain was severe. Unfortunately, our abdominal examination found discoloration of the left flank that we suspect as pancreatitis (see Figure 3). Subsequent laboratory tests showed an increase in amylase number 138 U / L and lipase 200 U / L with normal liver function. Serum cholesterol was normal. The inflammatory markers of CRP did not show an increase of 0.7 mg / L while procalcitonin was 0.36 ng / mL. Abdominal ultrasound results showed a slightly enlarged pancreas with a very hypoechoic structure (see Figure 2). At the current examination, we found a positive Ranson prognostic Ranson's score for acute pancreatitis for the variable age 83 years. However, after a 48-hour evaluation, there was a positive Ranson criterion for increased hematocrit, urea, and base excess deficit. Therefore, the prognosis of mortality is around 11-15%.



Figure 2. Ultrasonography of Pancreatitis.

Based on the results of the examination, the patient was diagnosed with COVID-19 and acute pancreatitis. Some of the most common etiologies of pancreatitis are etiologies such as gallstones, alcohol, pancreatitis-causing drugs such as furosemide, thiazide, sulfa, hypertriglyceridemia, other viral infections, autoimmune, posttraumatic were not found in this patient. Besides, the use of Clopidogrel might considerably be also one of the triggering factors since Lai et al. declared that persons actively consume Clopidogrel were at 8.46-fold increased odds for acute pancreatitis.²⁷ therefore, However, Tthe diagnosis of the cause of acute pancreatitis can be confirmed by autopsy to detect SARS-CoV2 in pancreatic cells. But we could not nt performed it because this examination was not done routinely.

However, we still manage patients according to the management of acute pancreatitis. At the time of the initial diagnosis of pancreatitis, the patient was fasting and receiving parenteral nutritional support, adequate fluid administration, also antibiotics according to culture results. The results of the patient's blood culture showed the growth of staphylococcus sp. then we changed the antibiotic treatment based on the results of the sensitivity test. Meanwhile, the patient has received Remdesivir as the anti-viral agent. Unfortunately, the patient falls into a state of septic shock then the patient died.



Figure 3. Abdominal appearance indicating suspicious acute pancreatitis.

DISCUSSION

COVID-19 often manifests as respiratory complaints, but extrapulmonary ones require extra attention. Recent studies show that gastrointestinal symptoms can reach 50% with symptoms such as nausea (17.3%), diarrhea (12.9%), anorexia (12.2%), abdominal pain (5.8%). Meanwhile, in a journal written by Wang et al, in a case series, nine people had acute pancreatitis along with COVID-19 infection. Liu et al wrote that 17% of the 67 cases of severe COVID-19 had acute pancreatitis, although only 7.46% of the pancreatic injury was able to be captured by computed tomography ^{14, 16}.

COVID-19 utilizes angiotensin-converting enzyme 2 (ACE2) as a receptor for the entry of viruses into human cells. ACE2 receptors are not only in the lungs but also widely spread in the esophageal, enterocyte, cardiovascular, renal, and pancreatic epithelial cells. Surprisingly, the amount of ACE2 RNA messenger was found more in the pancreas than in the lungs. ACE2 expression took both in the exocrine glands of the pancreas and in the islet cells. The spike protein (S) acts as a support for the ACE2 receptor. Expression of ACE2 and transmembrane serine protease 2 (TMPRSS2) that plays a vital role in the successful fusion of SARS-CoV2 into human cells, is found in β cells of the pancreas ⁷.

Acute pancreatitis is a condition when the pancreas is inflamed. This inflammatory process may be confined to the pancreatic or peripancreatic tissue. The causes of this AP vary while severity also is divided to different degrees ^{12, 17-19}. The most common risk factors for acute pancreatitis are gallbladder disease (often caused by cholelithiasis) and chronic alcohol consumption. Acute pancreatitis is defined as the presence of typical pancreatic abdominal pain, an increase in serum amylase/lipase more than three times of regular value, and ultrasound, CT, or MRI imaging findings support the diagnosis ¹⁹⁻²¹.

In these patients, we found no risk factors that could explain the development of acute pancreatitis. Some of the most common pancreatitis etiologies such as gallstones, alcohol,

pancreatitis-causing drugs such as furosemide, thiazide, sulfa, hypertriglyceridemia, other viral infections, autoimmune, posttraumatic. No medical history of the patient seemed to cause the pancreatitis, but the use of Clopidogrel might possibly trigger it. His past medical history and drugs does not support the variable causes of pancreatitis. Patients with acute pancreatitis often have positive blood culture results when a systemic infection is found, especially in patients who have previously undergone intra-biliaer procedures. The results of blood culture in the most common acute pancreatitis patients were *Escherichia coli* and *Klebsiella sp.* Those systemic infection could be fatal. In this patient we found his blood culture positive for *Staphylococcus sp* meaning the source of infection could be anywhere that precipitate the septic state. Therefore, it is consistent with several similar case reports that it is possible to injury the pancreas in a patient with COVID-19 ^{12, 19}. In fact, in several observational case-control studies on pancreatic injury, there was an increase in serum amylase/lipase as a marker of pancreatic damage in 8.5-17.3% of cases. Interestingly, pancreatic abnormalities have been more frequently noted in the sub-group of patients having severe COVID disease ^{17-19, 22}.

Several hypotheses suggest the occurrence of pancreatitis in COVID-19, namely the expression of ACE2 in the pancreatic ductal, acinar, and islet cells so that the virus can easily spread from the duodenal epithelium to the pancreatic gland ^{17, 23, 24}. Other studies have shown that SARS-CoV-2 is able to infect pancreatic-induced pluripotent stem cells (iPSC) thus they produce proinflammatory cytokines such as CXCL12, IL-6, IL-8, IL-10 ¹². They observed that the SARS-CoV-2 hijacked the ribosomal machinery in the pancreatic cells and also increased the expression of some pancreatic ductal stress response genes. Prominently, the genes CXCL12, NFKB1, and STAT3 showed significant upregulation as compared to the control. The researchers report that the transcriptional analysis of SARS-CoV-2 infected iPSC-derived pancreatic cultures demonstrated active viral replication and pancreas-specific COVID-19 associated disease signatures. The SRP-protein targeting processes were upregulated, indicating

that host cell machinery was being repurposed for viral replication^{25,26}.

From RNA-sequencing studies, they established that the pancreas, specifically the exocrine compartment (acinar and ductal cells), has a high expression of ACE2. Gender and age present no difference in the expression of the ACE2 receptors. The researchers demonstrated that the iPSC-derived pancreatic cells used in this study exhibit ACE2 and TMPRSS2 expression. Both the receptors are present in the pancreas, especially in the exocrine portion²⁶.

Despite the many published cases of both the coincidence of acute pancreatitis with COVID-19, acute pancreatitis caused by COVID-19 is still unproven. However, if there is a case of acute pancreatitis with COVID-19, the occurrence of idiopathic acute pancreatitis due to COVID-19 cannot be neglected¹².

CONCLUSION

Cases of acute pancreatitis with COVID-19 can be a coincident or idiopathic. Thus far, diagnostic tool of this case is necrosis autopsy and SARS-CoV2 PCR. Through our observation, COVID-19 has raised suspicions of acute idiopathic pancreatitis in severe COVID-19. Therapy and monitoring in patients are still carried out according to the management of COVID-19 and pancreatitis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

1. Kumar M, Taki K, Gahlot R, Sharma A, Dhangar K. A chronicle of SARS-CoV-2: Part-I -Epidemiology, diagnosis, prognosis, transmission and treatment. *Science of The Total Environment*. 2020;734:139278.
2. Worldometer. COVID-19 Coronavirus Pandemic Data 2020 [cited 2020 01-02-2021]. Available from: <https://www.worldometers.info/coronavirus/>.
3. Liu Y, Ning Z, Chen Y, Guo M, Liu Y, Gali NK, et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*. 2020;582(7813):557–60.
4. Zhan M, Qin Y, Xue X, Zhu S. Death from Covid-19 of 23 Health Care Workers in China. *N Engl J Med*. 2020.
5. Hu Y, Deng H, Huang L, Xia L, Zhou X. Analysis of Characteristics in Death Patients with COVID-19 Pneumonia without Underlying Diseases. *Acad Radiol*. 2020;27(5):752.
6. Zhang B, Zhou X, Qiu Y, Feng F, Feng J, Jia Y, et al. Clinical characteristics of 82 death cases with COVID-19. *medRxiv*. 2020:2020.02.26.20028191.
7. Gupta N, Ish P, Kumar R, Dev N, Yadav SR, Malhotra N, et al. Evaluation of the clinical profile, laboratory parameters and outcome of two hundred COVID-19 patients from a tertiary centre in India. *Monaldi Archives for Chest Disease*. 2020;90.
8. Gupta S, Parker J, Smits S, Underwood J, Dolwani S. Persistent viral shedding of SARS-CoV-2 in faeces - a rapid review. *Colorectal Dis*. 2020;22(6):611–20.
9. Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Military Medical Research*. 2020;7(1):11.
10. Seeliger B, Philouze G, Cherkaoui Z, Felli E, Mutter D, Pessaux P. Acute abdomen in patients with SARS-CoV-2 infection or co-infection. *Langenbeck's Archives of Surgery*. 2020;405:861-6.
11. Huang JT, Ran RX, Lv ZH, Feng LN, Ran CY, Tong YQ, et al. Chronological Changes of Viral Shedding in Adult Inpatients With COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020;71(16):2158–66.
12. de-Madaria E, Capurso G. COVID-19 and acute pancreatitis: examining the causality. *Nat Rev Gastroenterol Hepatol*. 2021;18(1):3–4.
13. Ahmed A, Fisher JC, Pochapin MB, Freedman SD, Kothari DJ, Shah PC, et al. Hyperlipasemia in absence of acute pancreatitis is associated with elevated D-dimer and adverse outcomes in COVID 19 disease. *Pancreatolology : official journal of the International Association of Pancreatolology (IAP) [et al]*. 2021.

14. Rotar O, Khomiak I, Nazarchuck M, Rotar V, Khomiak A, Taneja K, et al. Utility of Presepsin for Diagnosis of Infected Acute Necrotizing Pancreatitis. *Journal of the Pancreas*. 2019;21(6):167–71.
15. Kariyawasam JC, Jayarajah U, Riza R, Abeysuriya V, Seneviratne SL. Gastrointestinal manifestations in COVID-19. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2021.
16. Wifi M-N, Nabil A, Awad A, Eltatawy R. COVID-induced pancreatitis: case report. *The Egyptian Journal of Internal Medicine*. 2021;33(1):10.
17. Kumar V, Barkoudah E, Souza DAT, Jin DX, McNabb-Baltar J. Clinical course and outcome among patients with acute pancreatitis and COVID-19. *Eur J Gastroenterol Hepatol*. 2021;33(5):695–700.
18. Bulthuis MC, Boxhoorn L, Beudel M, Elbers PWG, Kop MPM, van Wanrooij RLJ, et al. Acute pancreatitis in COVID-19 patients: true risk? *Scand J Gastroenterol*. 2021;56(5):585–7.
19. AlHarmi RAR, Fateel T, Sayed Adnan J, AlAwadhi K. Acute pancreatitis in a patient with COVID-19. *BMJ Case Rep*. 2021;14(2).
20. Sandhu H, Mallik D, Lokavarapu MJ, Huda F, Basu S. Acute Recurrent Pancreatitis and COVID-19 Infection: A Case Report with Literature Review. *Cureus*. 2021;13(2):e13490.
21. Wifi MN, Nabil A, Awad A, Eltatawy R. COVID-induced pancreatitis: case report. *Egypt J Intern Med*. 2021;33(1):10.
22. Narang K, Szymanski LM, Kane SV, Rose CH. Acute Pancreatitis in a Pregnant Patient With Coronavirus Disease 2019 (COVID-19). *Obstet Gynecol*. 2021;137(3):431–3.
23. Rasch S, Herner A, Schmid RM, Huber W, Lahmer T. High lipasemia is frequent in Covid-19 associated acute respiratory distress syndrome. *Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]*. 2021;21(1):306–11.
24. Troncone E, Salvatori S, Sena G, De Cristofaro E, Alfieri N, Marafini I, et al. Low Frequency of Acute Pancreatitis in Hospitalized COVID-19 Patients. *Pancreas*. 2021;50(3):393–8.
25. Samanta J, Gupta R, Singh MP, Patnaik I, Kumar A, Kochhar R. Coronavirus disease 2019 and the pancreas. *Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]*. 2020;20(8):1567–75.
26. Müller JA, Groß R, Conzelmann C, Krüger J, Merle U, Steinhart J, et al. SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. *Nature Metabolism*. 2021;3(2):149–65.
27. Lai S-W, Lin C-L, Liao K-F. Actively using clopidogrel correlates with an increased risk of acute pancreatitis in Taiwan. *Int J Cardiol*. 2015 Mar;183:263–6.

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Research Article

Soil-Transmitted Helminthes Infection and Nutritional Status of Elementary School Children in Sorong District, West Papua, Indonesia

Zukhaila Salma¹, Fitriah², Raden Bagus Yanuar Renaldy³, Lynda Rossyant⁴, IWayan Sarjana⁵, Soraya Salle Pasulu⁶, Budiono⁷, I Gusti Made Reza Gunadi Ranul⁸, Dominicus Husada⁸, Sukmawati Basuki^{2,4*}

¹Master of Tropical Medicine Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Laboratory of Malaria, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

³Bachelor of Medicine Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

⁴Department of Medical Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
Puskesmas Mayamuk, Sorong-98421, West Papua, Indonesia

⁵RSUD Kabupaten Sorong, Kampung Baru, Sorong, West Papua, Indonesia

⁷Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

⁸Department of Child Health, Dr. Soetomo Hospital/Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

It is known that soil-transmitted helminths (STHs) infection in children associates with growth and developed restriction in children, which is shown by nutritional status. However, the studies which are investigating this phenomenon is still limited in Indonesia. This recent study aimed to compare students who infected and non-infected with STH towards their nutritional status. An analytic cross-sectional research design was conducted in two elementary school students at Mayamuk sub-district, Sorong district, in January 2020. STHs infection was identified by lugol stained wet mount smear from their stool under a light microscope. Children nutritional status was determined by body mass index based on age. A total of 164 children (67.5%, 164/243) were voluntary to participate by informed consent and eligible. Twenty-seven children (16.5%, 27/164) were infected with one or more STH species of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, and *Strongyloides stercoralis*. *T. trichiura* (81.5%, 22/27) was the most common species found, either in single or mixed infection. Children nutritional status was observed as thinness, normal, overweight, and obese, that was 6.1% (10/164), 75% (123/164), 6.7% (11/164), and 12.2% (20/164) respectively. STHs infection occurred in children with nutritional status of thinness 3.7% (1/27), normal 74.1% (20/27), overweight 3.7% (1/27), and obese 18.5% (5/27). There was no significant difference between STHs infected children and non-infected children on their nutritional status ($p=0.616$, Chi-Square test). Thus, it indicated that STHs infection was not only the factor to induce the impairment of nutritional status in children at Mayamuk sub-district. It needs further investigation to clarify the factors which are leading to the thinness, overweight, and obese in Mayamuk children.

Keyword. Soil-transmitted helminthes infection; nutritional status; children; elementary school, Indonesia

ABSTRAK

Kecacingan yang ditularkan melalui tanah (infeksi STHs) pada anak telah diketahui mempengaruhi pertumbuhan dan perkembangan pada anak, yang ditunjukkan dengan status gizi. Penelitian yang membahas hal ini masih terbatas di Indonesia. Penelitian ini bertujuan untuk membandingkan anak yang terinfeksi STHs dengan anak yang tidak terinfeksi STHs terhadap status gizinya. Desain penelitian cross-sectional analitik dilakukan pada murid dari dua sekolah dasar pada bulan Januari 2020, di kecamatan Mayamuk, kabupaten Sorong. Identifikasi infeksi STHs menggunakan pemeriksaan mikroskopis dari sediaan tinja anak dengan metode wet mount smear yang tercatat oleh larutan lugol. Status gizi anak ditentukan dari indeks massa tubuh menurut usia. Sejumlah 164 anak (67,5%, 164/243) secara sukarela berpartisipasi

* Corresponding Author:
sukmab@fk.unair.ac.id

melalui informed consent dan sesuai kriteria. Dua puluh tujuh anak (16.5%, 27/164) terinfeksi oleh satu atau lebih spesies STHs, yakni *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, dan *Strongyloides stercoralis*. *T. trichiura* (81.5%, 22/27) merupakan spesies yang paling banyak ditemukan baik dalam infeksi tunggal maupun ganda. Status gizi anak yang didapatkan meliputi status gizi kurang (6,1%, 10/164), normal (75%, 123/164), gizi lebih (6,7%, 11/164) dan obesitas (12,2 %, 20/164). Infeksi STHs terjadi pada anak dengan status gizi kurang sebesar 3.7% (1/27), normal 74.1% (20/27), gizi lebih 3.7% (1/27), and obesitas 18.5% (5/27). Tidak ditemukan perbedaan yang bermakna antara anak yang terinfeksi STH dengan yang tidak terhadap status gizinya ($p=0.616$, uji Chi-Square). Hal ini menunjukkan bahwa infeksi STH bukan satu-satunya faktor penyebab gangguan terhadap status gizi anak di kecamatan Mayamuk. Kajian lebih lanjut perlu dilaksanakan untuk menentukan faktor penyebab status gizi kurang, gizi lebih, dan obesitas pada anak di kecamatan Mayamuk.

Kata kunci: Infeksi soil-transmitted helminthes; status gizi; anak; sekolah dasar, Indonesia.

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INTRODUCTION

Soil-transmitted helminthes (STHs) infection is one of the neglected tropical infectious diseases which commonly occur in low-income countries and rural communities. Helminths that cause STHs infection in humans, are *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale*^{1,2}. Pullan et al estimated that 1.45 billion people worldwide were infected with at least one species of these helminths in Asia³. Globally, an estimated disability-adjusted life years (DALYs) contributed by STHs infection was 1.9 million in 2017⁴.

STHs infection is a chronic infection that tends to be asymptomatic, thus it is difficult to assess the morbidity, especially in endemic area^{5,6}. Symptoms and signs of STHs infection are anorexia, anemia, dysentery, diarrhea, and intestinal obstruction which can affect the growth and development of the child. The presence of STHs in the small intestine can interfere the absorption of nutrients and cause intestinal bleeding⁵⁻¹⁰. Several studies showed that STHs infection was significantly associated with a decrease of nutritional status indicators involving weight for age and height for age^{10,11}. The severity of clinical manifestation is commonly performed by the infection with polyparasitism and heavy intensity of STHs^{6,7,12}.

STHs infection and stunting in children are public health problems in Indonesia. The

national survey showed that the average of STHs infection prevalence of elementary school students between 2000-2011 was 28.7%¹³. Several studies had indicated that STHs infection in elementary school students in rural areas of Indonesia were remained high¹⁴⁻¹⁶. The World Health Organization (WHO) reports that the cases number of under five year old children who experience wasting and stunting in 2019 were 47 million and 144 million children, respectively, and most of them founded in Africa and Asia^{17,18}. Riset Kesehatan Dasar Indonesia showed that the prevalence of wasting and stunting of children in 2018 were 10.2% and 30.8%, respectively¹⁹. In 2018, twenty out of thirty-four (58.9%, 20/34) provinces of Indonesia were categorized as high stunting prevalence province²⁰.

West Papua is one of the Indonesian provinces, which is facing these two health problems. A study showed that the STHs infection prevalence of elementary school children in the Sorong ditrict was 30.6%²¹. A National nutritional status survey in 2018 reported that the prevalence of school-age children and adolescents (5-12 year old) with stunting and wasting condition was 22.8% and 6.8%, respectively in West Papua²². Until now, it has not yet been studied the phenomenon of STHs infection with nutritional status in West Papua. Our study aimed to compare between children infected and non-infected with STHs towards their nutritional status. It would be meaningful for the control program of STHs infection and stunting.

MATERIALS AND METHODS

Study area and population

The study was conducted in two villages, where are located in Mayamuk sub-district, Sorong, West Papua Province, Indonesia, where the average temperature of area was 27,9⁰C and the humidity was 83,2%. Geographically, most of the Sorong area, a district, is directly adjacent to Indonesian sea areas. It is bordered by the Pacific Ocean to the North; Seram sea to the South and West; Tambrauw District to the East and Raja Ampat regency to the west. Sorong consists of 30 sub-districts and 115 islands with a total area of 13,075.28 km² (Figure 1). Distribution of Gross Regional Domestic Product in 2019 based on sectors comprised of processing industri (42.54%), addition and excavation (15.95%), construction (14.65%), agriculture, forestry, and fisheries (10,11%), and others (16.75%). The main production of the plantation sector in Sorong are coconut, coffee, and cocoa²³.

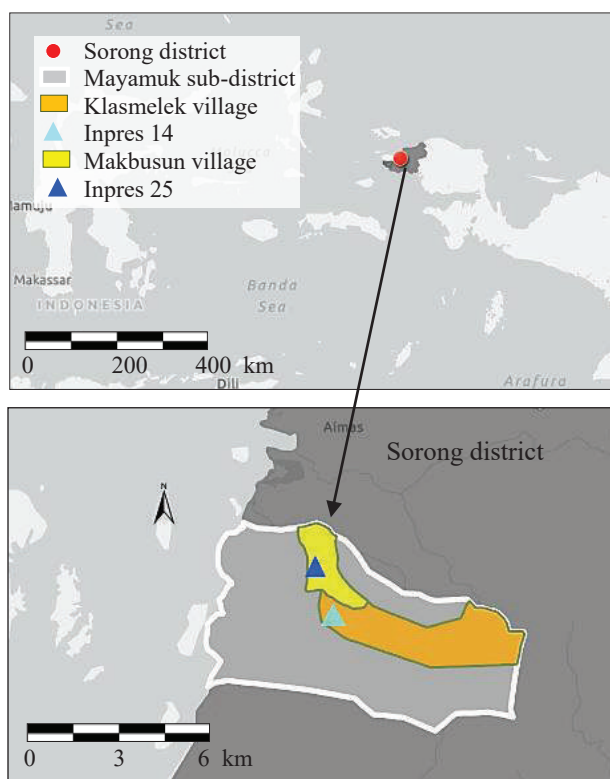


Figure 1. Study sites (source: arcgis.com²⁴).

Mayamuk sub-district represents 4.4% (542.2 of 13,693.5 km²) of the total area of Sorong.

Study was implemented in two public elementary schools, namely Inpres 14 and Inpres 25, in January 2020. Inpres 14 is located in Klasmelek village, while Inpres 25 is in Makbusun village. The distance between the two elementary schools is 3.1 kilometers. Makbusun village is located \pm 8.5 km from seashore, while Klasmelek village is located \pm 10.9 km from it. Plantations, forest areas, and rivers are many in Klasmelek village than in Makbusun village. Total of 3107 people are living in Makbusun village and 674 people are in Klasmelek village.

Sample and data collection

An analytical cross-sectional study design was conducted. Elementary school students from grade one to grade six from both schools involved in this study. The minimum number of samples was determined by the proportion estimation formula added 10% to anticipate error result and total was 90 samples. A structured questionnaire which included information on general demographic data (name, date of birth, age, gender, and ethnic), history of STHs infection, and anti-helminthic drug was used.

Stool collection and STHs identification

Children who participate in this research were given a stool tube (OneMed, Sidoarjo, Indonesia) which had labeled according to the questionnaire number. They brought the tube back with as much as one knuckle of stool on the next day. The stools in tube were preserved with adding 10% formalin solution and checked the tube number based on the questionnaire data. STHs was identified by using wet-mount smear method stained with 1% Lugol solution under light microscope with 100 and 400 magnifications (Olympus© CX22, Japan). It was repeated four times. Stool examination was performed in the Institute of Tropical Disease, Airlangga University, Surabaya.

Nutritional status measurement

The body mass index according to age (BAZ) score was used to determine nutritional status of children. It is based on the body weight, height, and age. The children body weight and height

were measured to complete their questionnaire form. A calibrated needle scale (OneMed, Sidoarjo, Indonesia) to the nearest 0.1 kg without shoes was used for measuring their body weight, and a microtoise (OneMed, Sidoarjo, Indonesia) to the nearest 0.1 cm which attached to a vertical wall was applied for sizing their height with barefeet. Their age was calculated in full month. Nutritional status was classified as severely thinness (<-3 standard deviation (SD)), thinness (-3 SD to <-2 SD), normal (-2 SD to $+1$ SD), overweight ($+1$ SD to $+2$ SD), and obese ($>+2$ SD)²⁵.

Statistical analyzes

Categorical variables were presented by number and percentage, while continuous variable was a mean value. The proportion differences of categoric variables were analyzed by *Chi-Square* test. Mean comparison of continuous variables were carried out by *t-test* analysis on normal distribution data and by *Mann-Whitney* test on abnormal distribution data. A significant comparison or difference was determined by $P<0.05$ value. All statistical analysis of this study was performed in version 22.0 Statistical Package for the Social Science (SPSS) (IBM, Somers, NY).

Ethical clearance

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga in number of 167/EC/KEPK/FKUA/2020.

RESULTS

Interview and anthropometric measurement were conducted into 194 children from two elementary schools, who were voluntary to participate in this study. A total of 164 children (84.5%, 164/194) were included and 30 children were excluded because they were without stools (Figure 2). Most of the children were non-Papuan (79.9%, 131/164) (Table 1).

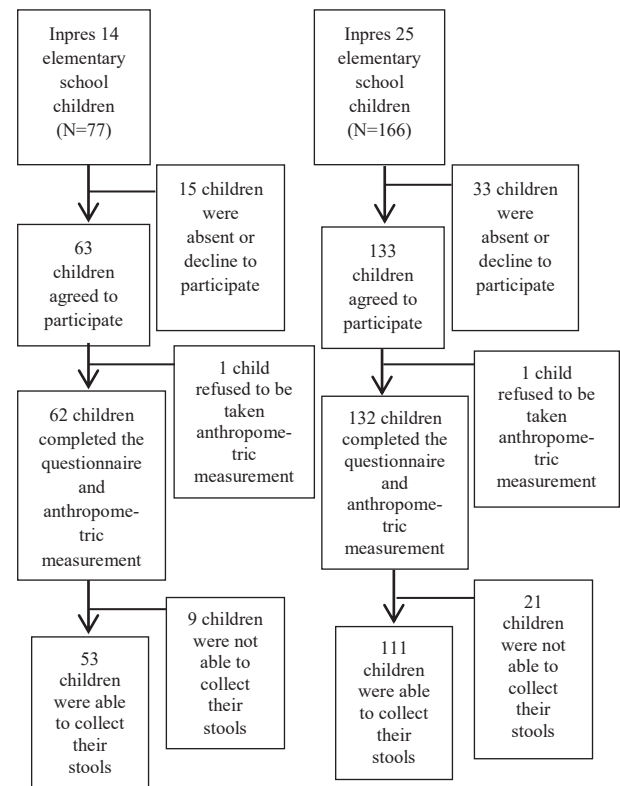


Figure 2. Diagram of participant involvement

Table 1. Demographic characteristic of children in Inpres 14 and Inpres 25 elementary school at Sorong District

Variable	Inpres 14 (n=53) (n, %)	Inpres 25 (n=111) (n, %)	Total (n=164) (n, %)
Age			
6 – 7	13, 24.5	26, 23.4	39, 23.7
8 – 9	22, 41.5	46, 41.4	68, 41.5
10 – 11	16, 30.2	34, 30.6	50, 30.5
12 – 13	2, 3.8	4, 3.6	6, 3.7
>13	0, 0.0	1, 1	1, 0.6
Sex			
Girl	20, 37.7	56, 50.4	76, 46.3
Boy	33, 62.3	55, 49.6	88, 53.7
Ethnic			
Papua	15, 28.3	18, 16.2	33, 20.1
Non Papua	38, 71.7	93, 83.8	131, 79.9

STHs were detected in 27 children stools (16.5%, 27/164). *T. trichiura* was frequently found

(13.4%, 22/164), then followed by *hookworm* (7.3%, 12/164) and *Ascaris lumbricoides* (3.6%, 6/164). Polyparasitized STHs were observed in

12 children stools (44.4%, 12/27) and dominated by *T. trichiura* with *hookworm* infection (50%, 6/12) (Figure 3 and Table 2).

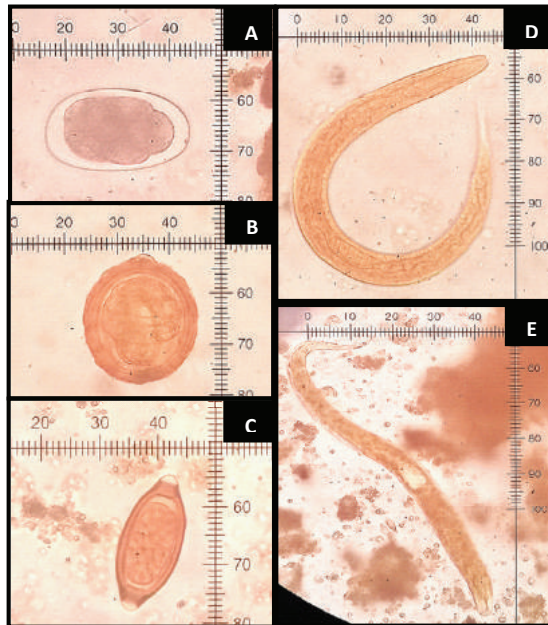


Figure 3. The morphology of soil-transmitted helminthes in children stools were (A) hookworm egg, (B) *A. lumbricoides* egg, (C) *T. trichiura* egg, (D) *S. stercoralis* larva and (E) hookworm larva under light microscope with 400 magnifications. Minimum length is 1 micrometer.

The majority of children had normal nutritional status (75%, 123/164). However, 41 children showed the abnormal nutritional status that

were 10 children with thinness (6.1%, 10/164), 11 children with overweight (6.7%, 11/164), and 20 children with obese (12.1%, 20/164). Children with thinness in Inpres 25 were higher than in Inpres 14 (7.2%, 8/111 v.s 3.7%, 2/53) (Table 3).

Table 2. Single and mix soil-transmitted helminthes infection cases among 29 infected children in Inpres 14 and Inpres 25 elementary school at Sorong District.

Variable	Inpres 14 (n=12) (n, %)	Inpres 25 (n=15) (n, %)	Total (n=27) (n, %)
Single infection	4, 33.3	11, 73.3	15, 55.6
AL	0, 0.0	1, 6.7	1, 3.7
TT	3, 25	7, 46.7	10, 37
HW	1, 8.3	1, 6.7	2, 7.4
SS	0, 0.0	2, 13.3	2, 7.4
Mix infection	8, 66.6	4, 26.7	12, 44.4
TT + AL	0, 0.0	2, 13.3	2, 7.4
TT + HW	6, 50	0, 0.0	6, 22.2
TT + HW + AL	1, 8.3	2, 13.3	3, 11.1
TT + HW + SS	1, 8.3	0, 0.0	1, 3.7

AL: *Ascaris lumbricoides*, HW: hookworm, SS: *Strongyloides stercoralis*, TT: *Trichuris trichiura*

Table 3. Characteristic of antropometric and nutritional measurements in children either with or without STHs infection at two elementary schools in Sorong District.

Antropometric and nutritional status	Elementary School						p-value ^a
	Inpres 14 N=53			Inpres 25 N=111			
	Positive	Negative	p-value ^a	Positive	Negative	p-value ^a	
Mean height (cm)	127.8	127.5	0.924	127	130.3	0.188	0.146
Man weight (kg)	27.1	27,6	0.890	27.8	28.3	0.952	0.253
Mean BMI	16.2	16.4	0.632	17	16.3	0.367	0.887
Mean BMI/age (z-score)	-0.1	-0,2	0.482	0.1	-0.2	0.587	0.808
Thinness (n, %)	0, 0	2, 4.9	0.598	1, 6.6	7, 7.2	0.183	0.511
Normal (n, %)	10, 83.4	33, 80.5		10, 66.7	70, 73		
Overweight (n, %)	1, 8.3	1, 2.4		0, 0	9, 9.4		
Obese (n, %)	1, 8.3	5, 12.2		4, 26.7	10, 10.4		

^a: Mann-Whitney test used for continous variable with abnormal data; T-test used for continous variable with normal data; Chi-Square test used for categorical data; Positive means children with STHs infection and negative is children without STHs infection

There was not significant difference between children who infected and non-infected with STHs towards their nutritional status ($p > 0.05$, *Chi-square*, test) (Table 4).

Table 4. Comparison of antropometric measurement in elementary children with and without STHs infection

Antropometric and nutritional status	STH infection Status		p-value
	Positive N=27	Negative N=137	
Mean height (cm)	127.3	129.5	0.299
Mean weight (kg)	27.5	28.1	0.957
Mean BMI	16.6	16.3	0.326
Mean BMI/age (z-score)	0.3	-0.19	0.397
Thinness (n, %)	1, 3.7	9, 6.6	0.616
Normal (n, %)	20, 74.1	103, 75.2	
Overweight (n, %)	1, 3.7	10, 7.3	
Obese (n, %)	5, 18.5	15, 10.9	

α : Mann-Whitney test used for continuous variable with abnormal data; T-test used for continuous variable with normal data; Chi-Square test used for nominal data; Positive means children with STHs infection and negative is children without STHs infection

DISCUSSION

School-age children living in a rural and a tropic area are vulnerable to STHs infection due to their habits and inadequate sanitation. School-age children often play in the ground without using footwear, rarely cut their nails, and do not wash their hands after playing or defecation^{26,27}. The potential factors for STHs infection in school-age children were due to their low hygiene practice.

A low prevalence of STHs infection was observed in this study (16.5%) based on WHO classification and a decline prevalence compared to previous prevalence in 2017²¹. Both studies were conducted in Mayamuk sub-district with different conditions. The previous study was performed in 2017, a year before lymphatic filariasis MDA implementation in Sorong district that is every October since 2018²⁸, and the recent study was 3 months after administration and two-year implementation of lymphatic filariasis MDA. It seemed that lymphatic filariasis MDA is able to reduce the STHs infection prevalence after 3 months administration and two-year

implementation of lymphatic filariasis MDA. Therefore, it might need the follow-up study in order to clarify the effect of lymphatic filariasis-MDA to reduce the STH prevalence.

A single dose of combination diethyl carbamazine (DEC) 100 mg and albendazole (ALB) 400 mg, a lymphatic filariasis MDA, is applied in Indonesia, including Sorong district^{29,30}. This combination has been reported that impacted to STHs infection, since the drugs have a broad range of anti-helminthic activity. It reduced 77% hookworm infection using the combination of ivermectine (IVM) and ALB in Côte d'Ivoire from 2014 to 2017³¹. Study by Sunish *et al* showed 79% reduction of STHs infection after 7 years administration the combination of DEC and ALB, and the highest reduction was for hookworm infection, followed by ascariasis, and trichuriasis³². Our study demonstrated the decline prevalence of STHs infections after 3 months administration and two-year implementation of DEC and ALB, but it was not under 10% of prevalence and it was still 46% reduction. It suggested that the health education to improve the individual hygiene and sanitation needs to be implemented in these areas. It could be considered to administer an additional single dose of ALB at six months before lymphatic filariasis-MDA in order to eliminate the STHs infection in children.

Infection of *T. trichiura* was highly found in this study, either within mixed, mostly *T. trichiura* with hookworm, or single infection. The previous study conducted in Sorong district reported similar results²¹. Studies in Côte d'Ivoire³¹, Tamil Nadu State³², and Congo³³ resulted a low reduction of trichuriasis compared with hookworm infection and ascariasis after lymphatic filariasis MDA administration by using respectively IVM-ALB, DEC-ALB, and alone ALB. It means that either those combinations or ALB alone by a single dose are not enough effective to eliminate *T. trichiura* infection in human.

The present study found no significant difference between STHs infected children and non-infected children toward their nutritional status. It was similar with the previous studies, which had been conducted by Suraweera *et al.* in

Kandy, Sri Lanka and Kurniati *et al.* in Madura, Indonesia^{34,35}. We found that the thinnest children mostly were not infected with STHs infection (see on table 3 and 4). It indicated that nutritional status of children can be influenced by several factors, such as food intake, environment, ages, dietary habit and the type of food consumed, additional STHs infection^{36,37}. A study in Surakarta showed that school-age children with stunting were influenced by their poor energy and protein intake. These intakes were significantly related to the level of education and occupations of their mother and family income³⁸. The prevalence of undernutrition in children from low socio-economic family was found to be higher than those from middle- to upper- socio-economic family (42.3% vs 19.28%)³⁹. The factors that underlie the low nutritional status within low-income family group are poverty, education of mother, number of family member, and also insecurity and safety of the food^{39,40}. Recently, the altered gut microbiota is associated with stunting and malnutrition in children^{41,42}. Thus, further investigation is needed to clarify the factors, which contribute to children thinness, overweight, and obese in Mayamuk sub-district, such as socio-economy, nutrient consumption, education, and gut microbiota, in order to overcome children nutritional status problem.

CONCLUSION

Children either with or without STHs infection did not have a significant difference in their nutritional status in Mayamuk sub-district. STHs infection was not the only factor leading to nutritional status impairment of children in this study. Thus, further research is needed to determine the factors, which affect to thinness, overweight, and obese in children living at Mayamuk sub-district, Sorong district, West Papua province.

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CONFLICT OF INTEREST

All authors stated that there is no conflict of interest exists.

REFERENCES

1. WHO. Soil-transmitted helminth infections, Fact sheet Updated March 2020. Available from: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>, accessed on May 26, 2020
2. Silver ZA, Kaliappan SP, Samuel P, Venugopal S, Kang G, Sarkar R, Ajjampur SSR, Geographical distribution of soil-transmitted helminthes and the effects of community type in South Asia and South East Asia – A systematic review. *PLoS Negl Trop Dis* 2018;12(1):e0006153
3. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil-transmitted helminth infection in 2010. *Parasite&Vector* 2014;7(37)
4. Kyu HH, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, Abbastabar H, Abd-Allah F, Abdela J, Abdelalim A, *et al.* Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: asystematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; 392:1859-922
5. Usuanlele, MT. Soil-transmitted helminth infection, nutrition and growth in school-age children from rural communities in Honduras. Thesis. 2012. Master of Science in Applied Health Sciences, Faculty of Applied Health Sciences, Brock University, St. Catharines, Ontario.
6. WHO. Guideline: preventive chemotherapy to control soil-transmitted helminth infection in at-risk population groups. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
7. Crompton DWT and Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. *Annu. Rev. Nutr.* 2002;22:35-59.
8. Farhadi S and Ovchinnikov RS. The relationship between nutrition and infectious diseases: a review. *Biomed Biotechnol Res J.* 2018;2:168-72
9. Echazu A, Juarez M, Vargas PA, Cajal SP, Cimino RO, Heredia V, Caropresi S, Paredes G, Arias LM, Abril M,

- Gold A, Lammie P, Krolewiecki A. Albendazole and ivermectin for the control of soil-transmitted helminthes in an area with high prevalence of *Strongyloides stercoralis* and hookworm in northwestern Argentina: a community-based pragmatic study. *PLoS Negl Trop Dis.* 2017;11(10):1-20
10. Sanchez AL, Gabrie JA, Usuanlele MT, Rueda MM, Canales M, Gyorkos TW. Soil-transmitted helminth infections and nutritional status in school-age children from rural communities in Honduras. *PLoS Negl Trop Dis.* 2013;7(8): e2378
 11. Moncayo AL, Lovato R, Cooper PJ. Soil-transmitted helminth infections and nutritional status in Ecuador: findings from a national surveys and implication for control strategies. *BMJ Open.* 2018;8(4):1-9: e021319
 12. Mupfasoni D, Karibushi B, Koukounari A, Ruberanziza E, Kaberuka T, Kramer MH, Mukabayire O, Kabera M, Nizeyimana V, Deville MA, Ruzin J, Webster JP, Fenwick A. Polyparasite helminth infection and their association to anemia and under-nutrition in northern Rwanda. *PLoS Negl Trop Dis.* 2009;3(9): e517
 13. DITJEN P2PL. Profil: pengendalian penyakit dan penyehatan lingkungan. Jakarta: Direktorat Jendral Pengendalian Penyakit dan Penyehatan Lingkungan. 2015.
 14. Mau F and Mulatsih. Prevalence and intensity of soil-transmitted helminth infections among elementary school students in West Sumba and Central Sumba districts East Nusa Tenggara, Indonesia. *Journal of Medical Science and Clinical Research.* 2017;5(10).
 15. Pasaribu AP, Alam A, Sembiring K, Pasaribu S, Setiabudi D. Prevalence and risk factors of soil-transmitted helminthiasis among school children living in an agricultural area of North Sumatera, Indonesia. *BMC Public-Health.* 2019;19(1):1066.
 16. Brahmantya IBY, Iqra HHP, Hartawan IGNBRM, Anjani IAW, Sudarmaja IM, Ryalino C. Risk factors and prevalence of soil-transmitted helminth infections. *Open Access Macedonian Journal of Medical Science.* 2020;8(A):521-24.
 17. WHO. Malnutrition, Fact Sheet, Updated April 2020. Available from: <https://www.who.int/news-room/fact-sheets/detail/malnutrition>, accessed on October, 2020
 18. UNICEF, WHO, Worl Bank Group. Levels and trends in child malnutrition: UNICEF/WHO/World Bank Group joint child malnutrition estimates, key finding of the 2020 edition. 2020. Geneva: WHO. Licence: CC BY-NC-SA 3.0 IGO
 19. Kementerian PPN. Pembangunan gizi di Indonesia. 2019. Jakarta: Direktorat Kesehatan dan Gizi Masyarakat-Kepedulian Pembangunan Manusia, Masyarakat dan Kebudayaan-Kementerian PPN/Bappenas.
 20. WHO. Nutrition landscape information system (NLIS), Help Topic: malnutrition in children, stunting, wasting, overweight, and underweight. Available from: <http://apps.who.int/nutrition/%0Alandscape/help.aspx?menu=0&helpid=391&lang=EN>, accessed on October 2020.
 21. Yuwono N, Pasulu SS, Husada D, Basuki S. Prevalence of soil-transmitted helminthiasis among elementary children in Sorong district, West Papua. *Indonesian Journal of Tropical and Infectious Diseas.* 2019;7(4):86-91
 22. Kemenkes RI. Buku saku: hasil pemantauan status gizi (PSG) tahun 2017. 2018. Jakarta: Direktorat Gizi Masyarakat-Direktorat Jendral Kesehatan Masyarakat-Kementerian Kesehatan.
 23. BPS Kabupaten Sorong. Kabupaten Sorong dalam angka: 2020. 2020, Kabupaten Sorong: Badan Pusat Statistik. ISSN: 2302-0512. Publication number: 91070.2003.
 24. ArcGIS. Available from: <https://www.arcgis.com/home/signin.html?returnUrl=https%3A//www.arcgis.com/home/item.html%3Fid%3D92be9dc23fa14a2e83a8bc4a6f7caeba>, accessed on October 2020.
 25. Peraturan Menteri Kesehatan RI. 2020. Permenkes RI No.2 Tahun 2020 tentang Standar Antropometri Anak.
 26. Wiryadana KA, Putra IWAS, Rahayu PDS, Pradnyana MM, Purwanta MLA, Sudarmaja IM. Risk factors of soil-transmitted helminth infection among elementary school students. *Paediatrica Indonesia.* 2017;57(6): 295-302.
 27. Suryantari SAA, Satyarsa ABS, Hartawan IGNBRM, Parastuta IKY, Sudarmaja IM. Prevalence, intensity and risk factors of soil-transmitted helminths infections among elementary school students in Ngis village, Karangasem district, Bali. *Indonesian Journal of Tropical and Infectious Disease.* 2019;7(6):137-143.
 28. Budijanto, D. (Maret, 2021). Kebijakan Program Pencegahan dan Pengendalian Penyakit Tular Vektor dan Zoonotik. Slide dipresentasikan di Seminar Daring Nasional P2PTVZ Kemenkes, Jakarta.
 29. Arianto MF, Kadir AR, Maria IL. Pelaksanaan program eliminasi filariasis di kota Sorong. *Tunas-Tunas Riset Kesehatan.* 2020;10(1).
 30. Kemenkes RI. Peraturan Menteri Kesehatan Republik Indonesia nomor 94 tahun 2014 tentang penanggulangan filariasis. 2014. Jakarta: Kementerian Kesehatan Republik Indonesia.
 31. Loukouri A, Meite A, Koudou BG, Goss CW, Lew D, Weil GJ, *et al.* Impact of annual and semi-annual mass drug administration for lymphatic filariasis and onchocerciasis on hookworm infection in Cote d'Ivoire. *PLoS Negl Trop Dis.* 2020;14(9): e0008642.
 32. Sunish IP, Rajendran R, Munirathinam A, Kalimuthu M, Kumar VA, Nagaraj J, Tyagi BK. Impact on prevalence of intestinal helminth infection in school children administered with seven annual rounds of diethyl carbamazine (DEC) with albendazole. *Indian J Med Res.* 2015;141:330-39.

33. Pion SDS, Chesnais CB, Uvon NPA, Vlaminck J, Abdou A, Shako BK, Simuna GK, Tambwe JP, Weil GJ, Boussinesq M. The impact of years of semiannual treatments with albendazole alone on lymphatic filariasis and soil-transmitted helminth infections: a community-based study in the Democratic Republic of the Congo. *PLoS Negl Trop Dis.* 2020;14(6): e0008322.
34. Suraweera O, Galgamuwa L, Wickramasinghe S, Iddawela D, Nandasiri N. Soil-transmitted helminth infections, associated factors and nutritional status in an estate community in Sri Lanka. *Sri Lankan Journal of Infectious Disease.* 2018;8(2):100-14.
35. Kurniati M, Budiono, Sulistyawati SW. Intestinal protozoa infection in relation to nutritional status of the Mandangin Island elementary school 6 students in Sampang regency. *Journal of Aesculap Medical Science.* 2019;10(1): 25-28.
36. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. *Parasitology.* 2000;121:S23-38.
37. Ulijaszek SJ. Relationships between undernutrition, infection, and growth and development. *Human evolution.* 1996;11:233-48.
38. Utami AD, Indarto D, Dewi YLR. The effect of nutrient intake and socioeconomic factor toward stunting incidence among primary school students in Surakarta. *Journal of Epidemiology and Public Health.* 2017;2(1);1-10.
39. Babar NF, Muzaffar R, Khan MA, Imdad S. Impact of socioeconomic factors on nutritional status in primary school children. *J Ayub Med Coll Abbottabad.* 2010;22(4):15-18.
40. Kamiya Y. Socioeconomic determinants of nutritional status of children in Lao PDR: effects of household and community factors. *Journal of Health, Population and Nutrition.* 2011;29(4):339-48.
41. Kumar M, Ji B, Babaei P, Das P, Lappa D, Ramakrishnan G, et al. Gut microbiota dysbiosis is associated with malnutrition and reduced plasma amino acid levels: Lessons from genome-scale metabolic modeling. *Metab Eng.* 2018; 49:128–42.
42. Vonaesch P, Rendremanana R, Gody JC, Collard JM, Giles-Vernick T, Doria M, et al. Identifying the etiology and pathophysiology underlying stunting and environmental enteropathy: study protocol of the AFRIBIOTA project. *BMC Pediatr.* 2018; 18(1):236

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Research Article

Increased Interleukin-6 as Inflammatory Response and Magnesium Deficiency in Pre-dialysis Chronic Kidney Disease of Indonesian Children

Astrid Kristina Kardani, Jusli Aras, Risky Vitria Prasetyo, Ninik Asmaningsih Soemyarso*, Mohammad Sjaifullah Noer
Department of Child Health, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital,
Surabaya, Indonesia

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ABSTRACT

Chronic kidney disease (CKD) is a serious health problem in children, with increasing morbidity and mortality rates throughout the world. Children with CKD tend to experience magnesium (Mg) deficiency that can stimulate an inflammatory response in the body. One of the inflammatory responses is an increase of Interleukin-6 (IL-6). Study to analyze the correlation between Mg and IL-6 in pre-dialysis CKD children. The methods a cross sectional study was conducted in Dr Soetomo General Academic Hospital from November 2018 to April 2019. Children with pre-dialysis CKD were included in this study. Variables of serum Mg level (mg/dL) and inflammatory marker (IL-6) were measured from the blood and analyzed by ELISA method. The correlation between Mg and IL-6 was analyzed with Spearman's correlation test with $p < 0.05$. Result a total of 47 children (27 boys vs 20 girls) between 3 months to 18 years old, with pre-dialysis CKD and no history of magnesium supplementation were included. The primary disease that causes of CKD were lupus nephritis (38.3%), nephrotic syndrome (23.4%), urologic disorder (23.4%), tubulopathy (10.6%) and others (4.3%). The average IL-6 level was 55.42 ± 43.04 pg/dL and Mg level was 2.06 ± 1.54 mg/dL. There were no significant correlation between IL-6 level and Mg level with staging of CKD and duration of illness ($p > 0.05$), but there was a significant correlation between serum Mg level and IL-6 level ($r = -0.748$; $p < 0.001$). Magnesium levels have a significant inverse correlation with IL-6 levels in pre-dialysis CKD children. The lower the Mg levels in the blood, the higher IL-6 levels and vice versa.

Keywords: Chronic kidney disease, Magnesium, Interleukin-6, Children, Elisa method.

ABSTRAK

Penyakit ginjal kronik (PGK) merupakan masalah kesehatan yang serius pada anak, dengan angka kesakitan dan kematian yang terus meningkat di seluruh dunia. Anak dengan CKD cenderung mengalami defisiensi magnesium (Mg) yang dapat merangsang respon inflamasi dalam tubuh. Salah satu respon inflamasi adalah peningkatan Interleukin-6 (IL-6). Penelitian untuk menganalisis hubungan antara Mg dan IL-6 pada anak PGK pra-dialisis. Metode penelitian cross sectional dilakukan di Rumah Sakit Umum Akademik Dr Soetomo dari November 2018 sampai April 2019. Anak-anak dengan CKD pra-dialisis diikutsertakan dalam penelitian ini. Variabel kadar Mg serum (mg/dL) dan penanda inflamasi (IL-6) diukur dari darah dan dianalisis dengan metode ELISA. Korelasi antara Mg dan IL-6 dianalisis dengan uji korelasi Spearman dengan $p < 0,05$. Hasil total 47 anak (27 laki-laki vs 20 perempuan) antara 3 bulan sampai 18 tahun, dengan CKD pra-dialisis dan tidak ada riwayat suplementasi magnesium dimasukkan. Penyakit utama penyebab PGK adalah lupus nephritis (38,3%), sindrom nefrotik (23,4%), kelainan urologi (23,4%), tubulopati (10,6%) dan lain-lain (4,3%). Rata-rata kadar IL-6 adalah $55,42 \pm 43,04$ pg/dL dan kadar Mg adalah $2,06 \pm 1,54$ mg/dL. Tidak terdapat hubungan yang bermakna antara kadar IL-6 dan kadar Mg dengan stadium PGK dan lama sakit ($p > 0,05$), namun terdapat hubungan yang bermakna antara kadar Mg serum dengan kadar IL-6 ($r = -0,748$; $p < 0,001$). Kadar magnesium memiliki korelasi terbalik yang signifikan dengan kadar IL-6 pada anak PGK pra-dialisis. Semakin rendah kadar Mg dalam darah, semakin tinggi kadar IL-6 dan sebaliknya.

* Corresponding Author:
niriksoemyarso@yahoo.com

Kata Kunci: Penyakit ginjal kronis, Magnesium, interleukin-6, Anak, Metode Elisa

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INTRODUCTION

Chronic kidney disease (CKD) is a serious health problem in adults and children, with increasing morbidity and mortality rates throughout the world¹. The number of CKD patients globally in 2019 was 13.4% (11.7 - 15.1%) of the world's population² with the amount in pediatric patients is quite high³. Moreover, the number of children with CKD in 2011 was 11-12 children per 1 million in CKD stages 3-5, .8 children per 1 million in CKD stages 3-5 (Europe), 5.7 children per 1 million (America), and 38 children per 1 million (Middle East and South Asia)³.

Chronic inflammation that occurs in CKD patients can increase the risk of disease becoming more severe and worsening of glomerular filtration rate¹. Children with CKD often have decreasing magnesium (Mg) levels. About 65% of Mg in the body is found in bones, 34% in smooth muscle and the remaining 1% is in plasma and interstitial fluid⁴. Magnesium deficiency can stimulate an inflammatory response and may influence the defense mechanism of human body⁵.

The inflammatory response in CKD patients is indicated by high levels of proinflammatory cytokines, including interleukin-6 (IL-6) that are associated with morbidity and mortality^{6,7}. Interleukin 6 is a dissolved IL-6 mediator with pleiotropic effect on inflammation, immune response, and hematopoiesis. Interleukin 6 is produced quickly and temporarily in response to infection and tissue injury, contributes to host defense through stimulation of acute phase responses, hematopoiesis, and immune reactions⁸.

Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, is the first referral hospital in East Java, Indonesia. The number of pediatric CKD patients in 2018 was 102 patients. Most patients were often being admitted to treatment ward with inflammatory problems as many as

72.4%. Based on the description above and limited studies, the researchers focusing on correlation between Mg and IL-6 levels in pre-dialysis pediatric CKD patients in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

METHODS AND MATERIALS

Participants

Participants in this study were children diagnosed with CKD⁹. The inclusion criteria were children aged 3 months to 18 years, diagnosed with CKD, in pre-dialysis. Children having received Mg supplementation and having an infection (fever/ temperature >37.5°C and high leukocyte levels) were excluded. Their parents were given an explanation regarding participant rights and obligations. All parents were also required to fill out an informed consent sheet.

Design

An analytic observational study was conducted using cross sectional design. The research was carried out at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, from November 2018 to April 2019 (figure 1). This research had been declared to meet ethical requirements by the Ethics Committee Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (0835/KEPK/XII/2018). Participants were chosen based on consecutive random sampling. The number of participants in this study were 47 participants. Participants were first identified for characteristics and then measured for Mg and IL-6 levels.

The examination of IL-6 and Mg levels were performed at Clinical Pathology laboratory of Dr. Soetomo General Academic Hospital. Measurement of IL-6 levels used ELISA test kits-Quantikine HS human IL-6 immunoassay (Elabscience Biotechnology Co., Ltd, Wuhan,

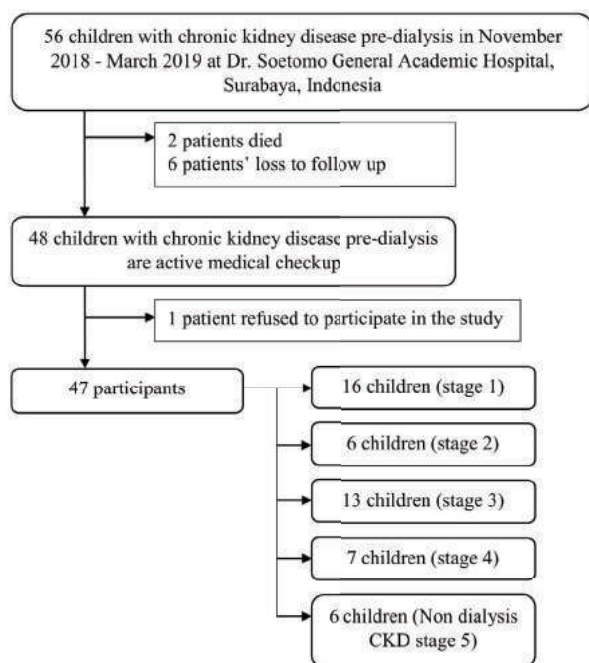


Figure 1. Participant recruitment process in this study

Hubei, China), during which participants were taken for venous blood. Blood samples were centrifuged at 3000 rpm for 15 minutes. The serum samples were collected and stored at -80°C in the laboratory of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The results of IL-6 measurement were categorized into 3 groups: high (≥ 4.5 pg/dL), normal (1.8-2.3 pg/dL), and low (< 1.8 pg/dL). Magnesium levels were measured using photometry method with EXL dimension analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). The measurement results were categorized into 3: group high (≥ 1.6 mg / dL), normal (1.2-1.6 mg / dL), and low (< 1.2 mg / dL)⁴.

Statistical Analysis

The results were displayed in figures and tables. The data were analyzed using IBM SPSS Statistics software version 22.0 (IBM Corp., Armonk, NY, USA). Analysis of correlation between Mg and IL-6 with staging of CKD and duration of illness, between Mg and IL-6 were carried out using Spearman correlation test. The $p < 0.05$ showed a significant correlation, while $p \geq 0.05$ indicated no significant correlation.

RESULTS

Characteristics of Participants

Table 1. Demographics Characteristics of Pre-dialysis CKD Children

Characteristics	n (%)
Sex	
Male	27 (57.45)
Female	20 (42.55)
Age (years)	
< 10 years	14 (29.79)
10-18 years	33 (70.21)
Duration of illness	
< 1 year	18 (38.30)
1-5 years	21 (44.68)
>5 years	8 (17.02)
Etiology	
Lupus Nephritis	18 (38.30)
Nephrotic Syndrome	11 (23.40)
Urological Disorders	11 (23.40)
Tubulopathies	5 (10.64)
HSP nephritis	2 (4.26)
CKD stage	
Stage I	16 (34.04)
Stage II	6 (12.77)
Stage III	12 (25.53)
Stage IV	7 (14.89)
Stage V	6 (12.77)
Mg	
Low	25 (53.19)
Normal	16 (34.04)
High	6 (12.77)
IL-6	
Low	4 (8.51)
Normal	0 (0.00)
High	43 (91.49)

CKD = Chronic kidney disease

Most children were boys of about 27 (57.45%) children. There were 33 children belonged to age group of 10-18 years (70.21%) (Table 1). The average age was 147.81 ± 55.20 months, while mean age of boy and girl were 140.89 ± 60.63 months and 157.15 ± 46.76 months, respectively. There were 21 (44.68%) children suffered from CKD for 1-5 years in, followed by children who experienced illness <1 year of about 18 (38.30%) children (Table 1). The average time of experiencing CKD was 30.64 ± 30.97 months. The average time of male and female participants duration of illness was 26.44 ± 20.67 months and 36.30 ± 40.97 months, respectively. The most common underlying disease in this study was lupus nephritis in 18 (38.30%)

children, followed by nephrotic syndrome and urological abnormalities, each occurred in 11 (23.40%) children (Table 1).

The Correlation between Magnesium and IL-6 Levels with staging of CKD

The Mg level in CKD stage 1 was 1.75±0,4 mg/dL, CKD stage 2 was 1.71±0,4 mg/dL, CKD stage 3 was 2.79±2.93 mg/dL, CKD stage 4 was 1.7±0.5 mg/dL and CKD stage 5 was 1.92±0,7 mg/dL (figure 2a). The IL-6 level in CKD stage 1 was 54±41.3 pg/dL, CKD stage 2 was 38.3±33.7 pg/dL, CKD stage 3 was 48.8±44.8 pg/dL, CKD stage 4 was 64.7±31.7 pg/dL and CKD stage 5 was 78.8±64.0 pg/dL (p=0.994, Figure 2b). There were no significant correlation between magnesium and IL-6 levels with staging of CKD (Figure 2a and 2b).

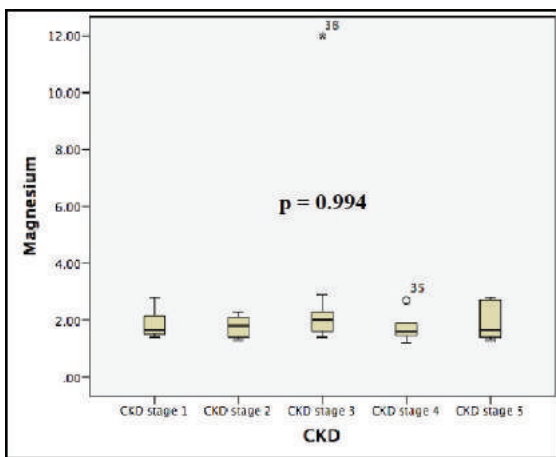


Figure 2a. Magnesium Level (mg/dL) Based on Staging of CK

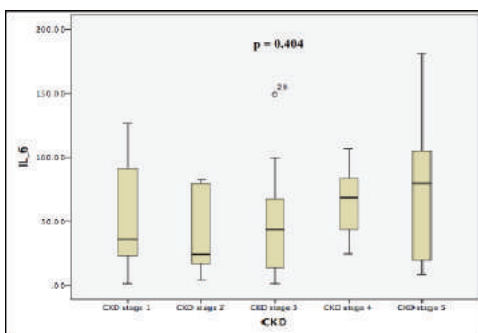


Figure 2b. IL-6 Level (pg/dL) Based on Staging of CKD

The average figure Ff There were no significant correlation between magnesium and IL-6 levels with staging of CKD (Figure 2a and 2b).

The Correlation between Magnesium an IL-6 Levels with Duration of Illness

The average Mg level in <1 year duration of CKD was 1,8±0,53 mg/dL, 1-5 years was 2,15±2,06 mg/dL, and > 5 years was 1,8±0,34 mg/dL (Figure 2c). The average IL-6 level in < 1 year duration of CKD was 53,6±46,3 pg/dL, 1-5 years was 59,6±41,6 pg/dL, and > 5 years was 44,7±43,3 pg/dL (p=0.883, Figure 2d). There were no significant correlation between magnesium and IL-6 levels with the duration of illness (Figure 2c and 2d).

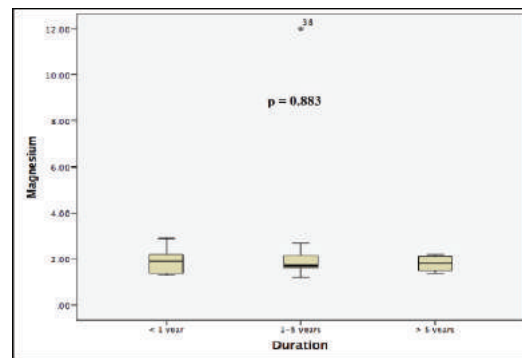


Figure 2c. Magnesium Level (mg/dL) Based on Duration of CKD

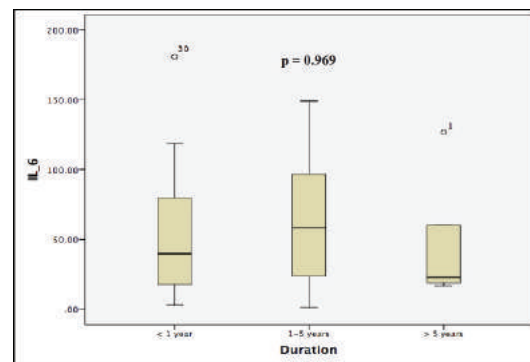


Figure 2d. IL-6 Level (pg/dL) Based on Duration of CKD

Correlation between Magnesium and IL-6 Levels

The average IL-6 level was 55.42±43.04 pg/dL, that was categorized into high IL-6 level. The average IL-6 level in boys and girls were 63.89±46.82 pg/dL and 43.98±35.30 pg/dL, respectively. Most children had high IL-6 levels in 43 (91.49%) children (Table 1). The average Mg level in boys and girls were 2.17±2.01 mg/dL and 1.90±0.46 mg/dL, respectively. Most participants had low Mg level in

25 participants (53.19%), followed by normal Mg in 16 participants (34.04%; Table 1).

The distribution of data was abnormal. The results of statistical analysis using Spearman correlation test showed a significant relationship between average Mg levels and average IL-6 levels ($p < 0.001$), with correlation coefficient value of -0.748 . This indicated a strong negative correlation between average Mg and average IL-6 levels in the blood. The lower Mg levels will further increase IL-6 levels as a proinflammatory mediator and vice versa (Figure 3a). There were no significant correlation between average of Mg ($p = 0.994$) and IL-6 levels ($p = 0.404$) with staging of CKD (Figure 3b and 3c), between average of Mg ($p = 0.883$) and IL-6 ($p = 0.969$) with duration of illness (Figure 3d-e).

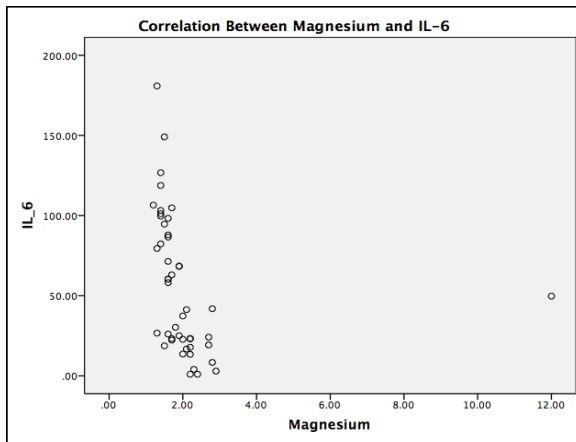


Figure 3a. Correlation between Mg (mg/dL) and IL-6 (pg/dL) in Pre-dialysis CKD Children ($r = -0.748$; $p = 0.001$)

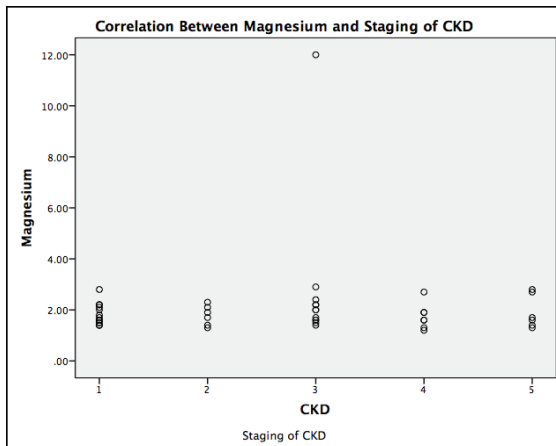


Figure 3b. Correlation between Mg (mg/dL) and Staging of CKD ($r = 0.01$; $p = 0.994$)

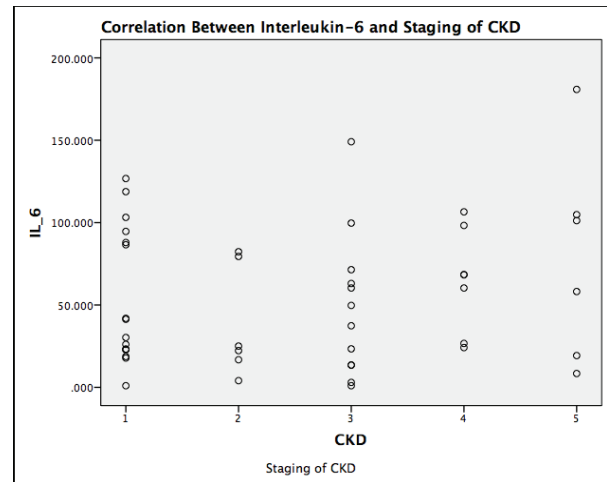


Figure 3c. Correlation between IL-6 (pg/dL) and Staging of CKD ($r = 0.125$; $p = 0.404$)

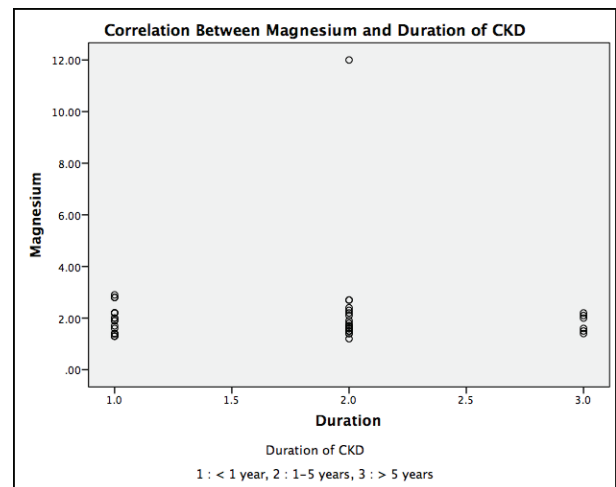


Figure 3d. Correlation between Magnesium (mg/dL) and duration of CKD ($r = -0.22$; $p = 0.883$)

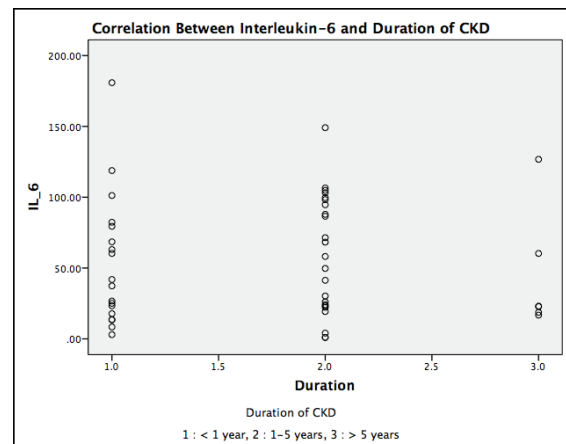


Figure 3e. Correlation between IL-6 (pg/dL) and duration of CKD ($r = -0.006$; $p = 0.969$)

DISCUSSION

This study found that most participants had low Mg level $<1,8$ mg/dL. Similar findings were obtained in Swaminathan's study [10], in which CKD patients with hypomagnesemia were higher than normal or high Mg levels. In stage 1-3 CKD patients, there is an increase in fractional Mg excretion as compensation for decreased or loss of kidney function to maintain normal serum magnesium levels in the blood. In CKD with Glomerular Filtration Rate (GFR) <10 ml/min/1.73m², this compensatory mechanism is less effective (insufficient) to prevent an increase Mg levels in the blood⁵. One of the causes of hypomagnesemia in this study might be caused by calcineurin inhibitors (cyclosporine) administration as a sparing agent for steroid therapy in children with resistant steroid nephrotic syndrome. Visscer *et al.*'s study⁵ stated that hypomagnesemia can be caused by drugs such as thiazide group, proton pump inhibitors, antibiotics, aminoglycoside groups and calcineurin inhibitors. Hypomagnesemia in CKD patients can also be caused by impaired intestinal absorption of Mg due to vitamin D deficiency, therefore routine vitamin D testing is needed in children with CKD^{11,12}. In addition, hypomagnesemia is one of the early predictors of the risk of cardiovascular and cardiovascular disease in CKD patients⁵.

The results of this study indicated that most participants experienced increasing IL-6 levels as one of the proinflammatory cytokines. Increased levels of IL-6 in CKD patients are most often caused by increased oxidative stress activity, chronic inflammation and fluid overload¹³. The decreased clearance of IL-6 results from impaired renal function¹⁴. Their study stated that increasing IL-6 levels in CKD patients were associated with the severity of metabolic acidosis and serum bicarbonate levels¹⁵. In addition, high levels of IL-6 are also caused by the activity of lupus nephritis and nephrotic syndrome^{14,16}.

Patients with nephrotic syndrome also have increased IL-6 levels. A study conducted by Subandiyah *et al.* found a significant increase in IL-6 levels in patients with steroid-resistant

nephrotic syndrome compared to steroid sensitive nephrotic syndrome¹⁷. Jafar *et al.*'s study obtained similar findings, stating that increasing IL-6 levels were found in patients with idiopathic nephrotic syndrome that was related to the therapeutic response¹⁸. Interleukin-6 expression in the urine and renal tissues was correlated with proteinuria in minimal changes disease rats¹⁹.

Cunningham *et al.* found that the quantitative excretion of magnesium tends to decrease in CKD stage 4 dan 5 and cannot be compensated by an increased fractional excretion of magnesium¹². In this study, there was no correlation between average magnesium level and staging of CKD, it might be due to several factors such as less of magnesium intake, calcineurin inhibitors (cyclosporine) administration and malabsorption in majority of children. Study by Magno *et al.* found the correlation of IL-6 dependent on the type of kidney disease and overlapping conditions such as hypertension and diabetes, but not by duration and staging of CKD. The measurement of IL-6 independently associated with mortality in patient with chronic kidney disease^{20,21,22}. It also can be used to explain that there were no correlation between average level of magnesium and IL-6 with duration of illness.

Statistical analysis showed a significant relationship between decreased Mg levels and increased IL-6 levels. Lower Mg level will cause higher IL-6 levels, which indicates a more severe inflammatory process. Measurement of Mg levels is affordable and can be used as an early predictor the severity of inflammatory process in pre-dialysis children with CKD. Magnesium is an important element that the body needs as a cofactor for >300 enzymatic reactions. Magnesium is needed for biochemical functions of various body metabolism pathways. Enzyme systems that involve magnesium include protein synthesis, muscle contraction, nerve function, controlling blood sugar, hormone receptor binding, regulating blood pressure, stimulating cardiovascular work, transmembrane ion flux, and connecting calcium. In addition, Mg has an important role in energy production in the body such as having a crucial role in the ATP metabolism (adenylate cyclase), oxidative phosphorylation, and glycolysis.

Another function of Mg is to play a role in the process of RNA and DNA synthesis^{23,24,25}. Whereas, IL-6 is a dissolved IL-6 mediator with pleiotropic effect on inflammation, immune response, and hematopoiesis⁸.

Kidneys have an important role in maintaining levels or concentrations of magnesium in the blood. The ability to regulate magnesium level will decrease along with decrease in kidney function. In addition, there is a decreased ability to absorb Mg in the intestine in CKD children when compared to normal children. The use of drugs as proton pump inhibitors (PPI) in CKD also reduces the ability of intestine to absorb magnesium. In patients who have undergone both hemodialysis and peritoneal dialysis, hypomagnesemia often results from the use of low-magnesium dialysate (low-Mg dialysate) fluids⁵.

CONCLUSION

Magnesium levels have a significant inverse correlation with IL-6 levels in pre-dialysis CKD children. Hypomagnesemia is associated with increased levels of IL-6 proinflammatory cytokines. Further research is needed to examine the role of magnesium with cardiovascular disease in children with CKD who do not yet have symptoms and signs.

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CONFLICT OF INTEREST

Astrid Kristina Kardani, Jusli Aras, Risky Vitria Prasetyo, Ninik Asmaningsih Soemyarso, and Mohammad Sjaifullah Noer declare that they have no conflict of interest this publication.

REFERENCES

1. Kaspar, C. D. W., Bholah, R., & Bunchman, T. E. A review of pediatric chronic kidney disease. *Blood purification*, 2016; 41(1-3), 211-217. doi:10.1159/000441737
2. Lv, J. C., & Zhang, L. X. (2019). Prevalence and disease burden of chronic kidney disease. *Renal Fibrosis: Mechanisms and Therapies*, 3-15. doi:10.1007/978-981-13-8871-2_1
3. Harambat J, van Stralen KJ, Kim JJ, Tizard EJ (2012) Epidemiology of chronic kidney disease in children. *Pediatric Nephrology*. 2012; 27 (3):363-373. doi:10.1007/s00467-011-1939-1
4. Floege J. Magnesium in CKD: more than a calcification inhibitor? *JNephrol*, 2015; 28 (3):269-277. doi:10.1007/s40620-014-0140-6
5. van de Wal-Visscher ER, Kooman JP, van der Sande FM (2018) Magnesium in Chronic Kidney Disease: Should We Care? *Blood purification* 45 (1-3):173-178. doi:10.1159/000485212
6. Hénaut L, Massy ZA. New insights into the key role of interleukin 6 in vascular calcification of chronic kidney disease. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, 2018; 33 (4):543-548. doi:10.1093/ndt/gfx379
7. Ferrè S, Li X, Adams-Huet B, Maalouf NM, Sakhaee K, Toto RD, Moe OW, Neyra JA. Association of serum magnesium with all-cause mortality in patients with and without chronic kidney disease in the Dallas Heart Study. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, 2018; 33 (8):1389-1396. doi:10.1093/ndt/gfx275
8. Tanaka T, Narazaki M, Kishimoto T (2014) IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 6 (10):a016295-a016295. doi:10.1101/cshperspect.a016295
9. Kidney Disease: Improving Global Outcomes CKD-MBDWG . KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl*, 2011; 7 (1):1-59. doi:10.1016/j.kisu.2017.04.001
10. Swaminathan R. Magnesium metabolism and its disorders. *Clin Biochem Rev* 24, 2003; (2):47-66
11. Sakaguchi Y, Hamano T, Isaka Y. Magnesium and Progression of Chronic Kidney Disease: Benefits Beyond Cardiovascular Protection?. *Advances in chronic kidney disease* 25, 2018; (3):274-280. doi:10.1053/j.ackd.2017.11.001
12. Cunningham J, Rodríguez M, Messa P. Magnesium in chronic kidney disease Stages 3 and 4 and in dialysis patients. *Clin Kidney J*. 2012; 5 (Suppl 1):i39-i51. doi:10.1093/ndtplus/sfr166
13. Su H, Lei CT, Zhang C. Interleukin-6 Signaling Pathway and Its Role in Kidney Disease: An Update. *Frontiers in immunology*, 2017; 8:405. doi:10.3389/fimmu.2017.00405

14. Jones, S. A., Fraser, D. J., Fielding, C. A., & Jones, G. W. Interleukin-6 in renal disease and therapy. *Nephrology Dialysis Transplantation*, 30(4), 564-574.2015; 30(4):564-574. doi:10.1093/ndt/gfu233
15. Zahed NS, Chehrazi S. The evaluation of the relationship between serum levels of Interleukin-6 and Interleukin-10 and metabolic acidosis in hemodialysis patients. *Saudi journal of kidney diseases and transplantation : an official publication of the Saudi Center for Organ Transplantation, Saudi Arabia*, 2017; 28 (1):23-29. doi:10.4103/1319-2442.198106
16. Cavalcanti A, Santos R, Mesquita Z, Duarte AL, Lucena-Silva N. Cytokine profile in childhood-onset systemic lupus erythematosus: a cross-sectional and longitudinal study. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas*, 2017; 50(4):e5738. doi:10.1590/1414-431x20175738
17. Subandiyah K, Ghofar HF, Fitri LE. Difference of Vitamin D and Interleukin-6 Levels in Children with Steroid-Resistant, Steroid-Sensitive and Idiopathic Nephrotic Syndrome. *Journal of Tropical Life Science*, 2019; 9 (2):179-187
18. Jafar T, Agrawal S, Mahdi AA, Sharma RK, Awasthi S, Agarwal GG. Cytokine gene polymorphism in idiopathic nephrotic syndrome children. *Indian J Clin Biochem*, 2011; 26 (3):296-302. doi:10.1007/s12291-011-0126-2
19. Kim SH, Park SJ, Han KH, Saleem MA, Lim BJ, Shin JI. (2016) Pathogenesis of minimal change nephrotic syndrome: an immunological concept. *Clin Exp Pediatr*, 2016; 59(5): 205-211. doi:10.3345/kjp.2016.59.5.205
20. Magno AL, Herat LY, Carnagarin R, Schlaich MP, Matthews VB. Current Knowledge of IL-6 Cytokine Family Members in Acute and Chronic Kidney Disease. *Biomedicines*, 2019; (7):1-15. doi :10.3390/2019/7010019.
21. Barreto DV, Barreto FC, Liabeuf S, Temmar M, Lemke HD, Choukron G, Massy ZA. (2010) Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease. *Kidney International*. 2010; 77:550-556. doi:10.1038/ki.2009.503
22. Fasset RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. (2011) Biomarkers in chronic kidney disease : a review. *Kidney International*, 2011; 80:806-821. doi:10.1038/ki.2011.198
23. Schwalfenberg GK, Genuis SJ. The Importance of Magnesium in Clinical Healthcare. *Scientifica (Cairo)* 2017; 4179326-4179326. doi:10.1155/2017/4179326
24. Patel H, Redkar V, Kulkarni A, Kale A. (2018) A study of serum magnesium level in patients with chronic renal failure at tertiary care hospital. *International Journal of Contemporary Medical Research*. 2018; 5(10);5-8. doi:10.21276/ijcmr.2018.5.10.21
25. Bressendorf I, Hansen D, Schou M, Silver B, Pasch A, Bouchelouche P, Pedersen L, Rasmussen LM, Brandi L. (2017) Oral magnesium supplementation in chronic kidney disease stages 3 and 4: efficacy, safety, and effect on serum calcification propensity, A prospective randomized double blinded placebo controlled clinical trial. *Kidney Int Rep*, 2017; 2: 380-389. doi:10.1016/j.ekir.2016.12.008

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Research Article

Antibacterial Activity of Ethanol Extract of Kemuning (*Murraya Paniculata*) Against *Klebsiella pneumoniae* ESBL by In Vitro Test

Illona Okvita Wiyogo¹, Pepy Dwi Endraswari², Yuani Setiawati³

¹Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

²Departement of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

³Departement of Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia

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ABSTRACT

Klebsiella pneumoniae Extended-spectrum β -lactamase (ESBL) was one of the microorganism that cause nosocomial infection which resistant to beta-lactams antibiotics. Orange Jessamine (*Murraya paniculata*) was traditional medicine which believed has antibacterial components, such as: flavonoids, alkaloids, essential oils, coumarins, terpenoids, tannins, and saponins. In the previous studies, there was antibacterial activity in ethanolic extract of *Murraya paniculata* againsts *E.coli*, *K.pneumoniae*, *S.typhi*, *E.faecalis*, *Paeruginosa*, *S.flexneri*, *S.aureus*, and *S.sonnei* with concentration 200 mg/mL. There has not experiment about ethanolic extract of *Murraya paniculata* against *Klebsiella pneumoniae* ESBL yet. The aim of this study was to find out the in vitro antibacterial activity of ethanol extracts of *Murraya Paniculata* against *Klebsiella pneumoniae* ESBL Broth dilution method with concentration 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, and 3,125 mg/mL were used for the determination of the Minimal Inhibitory Concentration (MIC). While the Minimal Bacterial Concentration (MBC) was assessed using streaking method in Nutrient Agar Plate. The highest concentration in this study was obtained from 100 g of *Murraya paniculata* leaves dissolved in 500 mL of 40% ethanol. The study was carried out 4 times replication. At the time of the sterility test extract, germ growth appeared on Nutrient Agar Plate media, so the extract was filtered before being used for research. After incubation at 37 °C for 24 hours, growth of bacterial colonies on all agar plates was observed. The concentration of the ethanol extract of *Murraya Paniculata* (200 mg/mL) did not inhibit the growth of *Klebsiella pneumoniae* ESBL. The ethanol extracts of *Murraya paniculata* in concentration 200 mg/mL had no antibacterial activity against *Klebsiella pneumoniae* ESBL.

Keywords: Antibacterial, ethanol extracts, *Klebsiella pneumoniae*, ESBL, *Murraya paniculata* leaves

ABSTRAK

Klebsiella pneumoniae extended-spectrum β -lactamase (ESBL) merupakan salah satu mikroorganisme yang menyebabkan infeksi nosokomial yang resisten terhadap antibiotik beta-laktam. Oranye Jessamine (*Murraya paniculata*) adalah obat tradisional yang diyakini memiliki komponen antibakteri, seperti: flavonoid, alkaloid, minyak esensial, kumarin, terpenoid, tanin, dan saponin. Dalam studi sebelumnya, ada antibakteri aktivitas ekstrak etanol *Murraya paniculata* melawan *E.coli*, *K.pneumoniae*, *S.typhi*, *E.faecalis*, *Paeruginosa*, *S.flexneri*, *S.aureus*, dan *S.sonnei* dengan konsentrasi 200 mg/mL. Belum ada eksperimen tentang ekstrak etanol *Murraya paniculata* terhadap *Klebsiella pneumoniae* ESBL. Tujuan dari penelitian ini adalah untuk mengetahui aktivitas antibakteri in vitro ekstrak etanol *Murraya Paniculata* terhadap *Klebsiella pneumoniae* ESBL. Metode pengenceran kaldu dengan konsentrasi 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12,5 mg/mL, 6,25 mg/mL, dan 3,125 mg/mL digunakan untuk penentuan Minimum Inhibition Concentration (MIC), sedangkan Minimal Bacterial Concentration (MBC) dinilai menggunakan metode goresan pada Pelat Agar Nutrien. Konsentrasi tertinggi dalam penelitian ini diperoleh dari 100 g daun *Murraya paniculata* yang dilarutkan dalam 500 mL etanol 40%. Penelitian dilakukan 4 kali replikasi, dimana pada saat ekstrak uji sterilitas pertumbuhan kuman muncul pada media agar nutrien agar; sehingga ekstrak disaring sebelum digunakan untuk penelitian. Setelah inkubasi pada 37 °C selama 24 jam, pertumbuhan koloni bakteri pada semua

* Corresponding Author:
illonawi@gmail.com

sehingga piring diamati. Konsentrasi ekstrak etanol *Murraya Paniculata* (200 mg/mL) tidak menghambat pertumbuhan *Klebsiella pneumoniae* ESBL. Ekstrak etanol *Murraya paniculata* dalam konsentrasi 200 mg/mL tidak memiliki aktivitas antibakteri terhadap *Klebsiella pneumoniae* ESBL.

Kata Kunci: Anti bakteri, Ekstrak Etanol, *Klebsiella pneumoniae*, ESBL, Daun *Murraya paniculata*

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INTRODUCTION

The percentage of nosocomial infection in Haji Adam Malik Hospital, Medan in 2010 is 6-16% and the mean is 9.8%.¹ The most frequently detected infection is Nosocomial Pneumonia (both ventilator and non-ventilator associated), and followed by urinary tract infection and central venous catheter associated bloodstream infections respectively.² Ventilator-associated Pneumonia (VAP) is the most common nosocomial infection among critical patients.

Nosocomial infection is handled differently from the non-nosocomial one since nosocomial infection is generally due to Multidrug-resistant bacteria. In developing countries, antibiotics are often used in irrational dose, hence the increased prevalence of antibiotic-resistant bacteria in hospitals.³ The prevalence of Imipenem-resistant *Acinetobacter*, Imipenem-resistant *P.aeruginosa*, and Oxacillin-resistant *S.aureus* are 67.3%, 27.2% and 82.1% respectively. Several bacteria are categorized as multidrug-resistant. The bacteria's high level of resistance might limit therapy options.⁴

Bacteria producing extended-spectrum β -lactamase (ESBL) cannot be overcome with penicillin, cephalosporin, and monobactam aztreonam, such as several *K. pneumoniae* strains.⁵ The infection case of *K. pneumoniae* ESBL in the group of nosocomial infection is 11 times higher than those of the community-acquired infection group.⁶

Indonesia is a tropical country with abundant medicinal plants, one of which is orange jessamine (*Murraya paniculata*). Medicinal plants generally contain phenolic compounds, i.e. phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, etc.⁷

The leaves of orange jessamine (*Murraya paniculata*) contain bioactive compounds which are secondary metabolites, such as alkaloids, flavonoids, saponins, terpenoids, and tannins.⁸ Since 1970, flavonoids and coumarins have been isolated from *Murraya paniculata*.⁹ Evaluations on the synthesis of nitro coumarins with or without the substitution of methyl or methoxy group has shown antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* strains.¹⁰

Other studies report that *Murraya paniculata* has many benefits including anti-platelet aggregation, anti-amoebic, anti-giardial, insecticide, pain relief, antidiabetic, antioxidant, antifungal, lipoxygenase and respiratory burst inhibitor.¹¹ This study aims to test antibacterial activity of orange jessamine extract against Multidrug-Resistance (MDR) bacteria, *Klebsiella pneumoniae* producing ESBL.

METHODS

This is a quasi-experimental study with Convenience Post Test Controlled Design, aimed to find out the antibacterial activity of the leaves of orange jessamine against *Klebsiella pneumoniae* ESBL with dilution method. The orange jessamine (*Murraya paniculata*) leaf extract were obtained from Materia Medica Laboratory, Batu, while antibacterial activity was tested in Microbiology Laboratory of Faculty of Medicine Universitas Airlangga.

The independent variable is ethanol extract of orange jessamine (*Murraya paniculata*) leaf extract concentration, while the dependent

variable is inhibition effect of *Klebsiella pneumoniae* ESBL bacteria growth in each tube containing concentration of ethanol extract orange jessamine leaf. The control variable are temperature and incubation time of sensitivity test with dilution method.

The suspension treatment groups of *Klebsiella pneumoniae* ESBL was exposed to ethanol extract orange jessamine leaf. The groups were T1 (100%), T2 (50%), T3 (25%), T4 (12.5%), T5 (6.25%), T6 (3.125%) and T7 (1.5625%). The control groups consisted of K1 (liquid medium and bacteria) and K2 (liquid medium and ethanol extract orange jessamine leaf). Minimum Inhibitory Concentration (MIC) is the lowest concentration that is still able to inhibit bacterial growth, Whereas, Minimum bactericidal concentration (MBC) is the lowest concentration that is able to kill bacteria. A further observation to determine MBC can be conducted upon the obtaining of growth inhibitory effect.

The test for antibacterial activity in ethanol extract orange jessamine leaf against *Klebsiella pneumoniae* ESBL is conducted with dilution method to determine MIC and MBC, which are analyzed descriptively and statistically using Analysis of Variance (Anova).

RESULT

This study used orange jessamine leaf extract obtained through maceration method. The highest concentration in this study was obtained from 100 g of *Murraya paniculata* dissolved in 500 mL of 40% ethanol. The compared extract concentrations were 200 mg/mL, 100 mg/mL, 50 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL

and 3.125 mg/mL. In this study, replications were carried out four times. During extract sterility test, there was bacterial growth in the medium Nutrient Agar Plates. Thus, the extract was filtered prior to the use in this study (Table 1).

The use of dilution method was aimed to assess the MIC. There were seven tubes with different concentration of extract ethanol of *Murraya paniculata* leaves and two tubes as the positive and negative control. The positive control tube contained bacteria and liquid Mueller Hinton Broth (MHB), while the negative one contained extract and liquid MHB. From all the conducted replications, all tubes were unable to identify as the extract color tended to appear dark (Figure 1). Thus, streaking was carried out on Nutrient Agar Plates to directly observe if there was any inhibition in the growth of *Klebsiella pneumoniae* ESBL bacteria by extract ethanol of *Murraya paniculata* leaves. After incubated in the temperature of 37°C for 24 hours, bacterial growth appeared in all Nutrient Agar Plates (Figure 2). The adding of extract up to the highest concentration showed that there was still bacterial growth in Nutrient Agar Plates

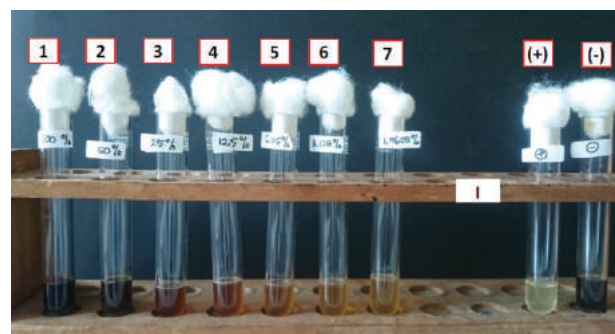


Figure 1. Results of dilution test of orange jessamine leaf extract using ethanol as solvent

Table 1. Data on Minimum Inhibitory Concentration showing no antibacterial effect on the extract concentration of 3.125-200 mg/mL.

Replication	Extract Concentration (mg/mL)						
	200mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-

Note: (-) bacterial growth appeared

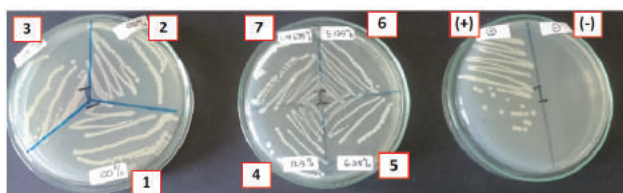


Figure 2. Results of streaking on Nutrient Agar Plates, showing bacterial growth in all extract concentration of *Murraya paniculata* leaf extract.

Analysis of the Results

This study on antibacterial activity used extract ethanol of *Murraya paniculata* leaves dissolved in 40% ethanol. This study was conducted by comparing the antibacterial activity of extract ethanol of *Murraya paniculata* leaves with ethanol as the solvent in several concentrations, ranging from 200-3.125 mg/mL. MHB media were put in all tubes (except for the one with 200 mg/mL concentration) to reduce the extract concentration to half of the initial concentration.

After all tubes were filled with the required amount of concentration, the bacterial suspensions measured using McFarland's 0.5 standard were added to each tube. Furthermore, the tubes were incubated in the temperature of 37°C for 24 hours. The results can be seen in tube 1 to 7 (the tubes appeared turbid, resembling the positive control tube). Similar thing occurred to the other tubes. Due to the turbidity, observation could not be carried out for MIC. Therefore, streaking was conducted on Nutrient Agar Plates to confirm the obtained results.

After incubated in 37°C temperature for 24 hours, there were bacterial colony growths in all Agar Plates. It was concluded that adding extract ethanol of *Murraya paniculata* leaves extract up to the concentration of 200 mg/mL is unable to inhibit the *Klebsiella pneumoniae* ESBL bacterial growth.

DISCUSSION

Dilution was carried out by preparing seven tubes containing MHB which then added with extract in certain concentrations and bacterial

suspension. After incubated in the temperature of 37°C for 24 hours, the tubes were observed. Bacterial growth still occurred if the solution appeared more turbid than in the negative control. The extract was made from 100 grams of *Murraya paniculata* and 500 mL of 40% ethanol. This study was carried out four times according to Federer's formula measurement. There were four mechanisms of bacterial resistance against β -lactam enzyme: β -lactam inactivation by β -lactamase enzyme; Penicillin Binding Protein (PBP) production with lower affinity against antibiotics; changes in porin channel leading to decreased permeability against antibiotics; and efflux pump that encourages antibiotics to escape the cells.¹²

Klebsiella pneumoniae ESBL is a bacterium that is able to product β -lactamase enzyme, the enzyme that resist to all penicillin and cephalosporins, including the sulbactam and clavulanic acid combinations and monobactams such as aztreonam.¹³ The expression of β -lactamase enzyme induced by mucopeptides, which is a product from cell wall metabolism of Gram-negative bacteria.¹⁴

According to the phytochemical assay, ethanol extract contains more secondary metabolite compounds than water extract does. Secondary metabolite compounds comprise alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins.¹⁵

Active ingredients of extract ethanol of *Murraya paniculata* leaves and are fathomed to have antibacterial effect are volatile oil, flavonoids, alkaloids, coumarins, terpenoids, saponins, and tannins. Volatile oil contains a compound acting as antibacterial by interrupting the forming of membranes or cell walls.¹⁶ Flavonoids, which is derived from phenol, show antibacterial activity since its penetration into cells causes protein precipitation, protein denaturation, protein coagulation, structure damage, and membrane lysis.¹⁷ Alkaloids interrupt peptidoglycan components in bacterial cells, causing cell walls not to form well and the cell itself to die.¹⁸ Coumarins show antibacterial activity due to its lipophilic structures and planar molecules that contribute to the penetration to cell

membrane or wall. Adding methyl or O-methyl group in the C6 or C7 position into coumarin aromatic core maintains the antibacterial activity in Gram-negative bacteria.²⁵

Terpenoids as a antimicrobial compounds whose mechanism of action is membrane disruption, could be futuristic biocide properties. It can be used in conjunction with other products such as antibiotics at sub-effective concentrations therefore it can confer bacterial resistance to antibiotics.¹⁹ Saponins extract of the *A. articulate* have antimicrobial activity on ranges of Gram- negative antibiotic-resistant isolates.²⁰ Saponin compound in *Acacia Arabica* extract has antimicrobial activity against diarrheagenic *E.coli*.²¹ Saponin-rich extracts from guar meal and quillaja exhibited antibacterial activity against *S.aureus*.²² Tannins has phenolic group which can be as antimicrobial and formulation based on tannin-rich plants have been used as diarrhea treatment.²³ The previous study proved antibacterial effect of *Murraya paniculata* extract. Ethanol extract in *Murraya paniculata* inhibits the growths of *E.coli*, *K.pneumoniae*, *S.typhi*, *E.faecalis*, *P.aeruginosa*, *S.flexneri*, *S.aureus*, and *S.sonnei* in 200 mg/mL concentration.⁽²⁴⁾ Meanwhile, 200 mg/mL concentration of ethanol extract in *Murraya paniculata* non-significantly inhibits *E.coli*, *P.mirabilis*, *S.Typhi* and *E.aerogenes*.²⁵

The results show that extract ethanol of *Murraya paniculata* leaves fail to inhibit and terminate *Klebsiella pneumonia* ESBL bacterial growth. This might be due to several matters, including: inhibition and termination of *Klebsiella pneumonia* ESBL require concentration of >200 mg/mL; combination with other antibiotics is required for optimum inhibition of *Klebsiella pneumonia* ESBL bacterial growth; further extraction is required until pure compound is obtained, enabling adjustment to optimum solvent.

In a study using *Murraya paniculata* ethanol extract with 300 mg/mL concentration, the growth of *E.coli*, *P.mirabilis*, *S.typhi*, dan *E.aerogenes* were inhibited significantly.²⁵ Other study reported that total alkaloids extracted from *Sophorea alpecuroides* L. combined with cefotaxime or

ceftazidime against *E. coli* ESBL has MICs of 12.5 mg/mL.²⁶ Total alkaloids increase bacterial susceptibility to cefotaxime and ceftazidime by 8-16 times. Natural flavonoid combined separately with amoxicillin. clavulanic acid, ampicillin/sulbactam and cefoxitin synergically inhibit the activities of *Klebsiella pneumoniae* ESBL that is still susceptible to imipenem and cefmetazole.²⁶

The three most studied coumarins include auraptene, umbelliprenin and 7-isopentenylcoumarin.²⁷ Auraptene inhibits bacterial activity producing β -lactamase class A.²⁸

In conclusion, The six flavonoids: 5,7-dimethoxyflavanone-4'-O- β -D-glucopyranoside; 5,7-dimethoxyflavanone-4'-O-[2''-O-(5'''-O-trans-cinnamoyl)- β -D-apiofuranosyl]- β -D-glucopyranoside; 5,7,3'-trihydroxy-flavanone-4'-O- β -D-glucopyranoside; naringenin 7-O- β -D-glucopyranoside; rutin; and nicotiflorin, inhibit the of *Klebsiella pneumoniae* ESBL growth.²⁹

CONCLUSION

Extract ethanol of *Murraya paniculata* leaves with concentration 200 mg/mL shows no antibacterial effects against the growth of *Klebsiella pneumoniae* ESBL. The MIC of orange jessamine leaf extract is indeterminable.

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REFERENCES

1. Jeyamohan D. Angka Prevalensi Infeksi Nosokomial Pada Pasien Luka Operasi Pasca Bedah Di Bagian Bedah Di Rumah Sakit Umum Pusat Haji Adam Malik, Medan Dari Bulan April Sampai September 2010. Microbiology and Management of Hospital Infection 2011.
2. Dasgupta, Sugata et al. Nosocomial Infections in the intensive care unit: Incidence, Risk Factors, Outcome and Associated Pathogens in A Public Tertiary Teaching Hospital of Eastern India. Indian Journal Critical Care Medicine. 2015;19(1):14-20.

3. Kuntaman. Analysis of Microbiology Results for Managing Hospital Acquired Infection Effectively Surabaya: Universitas Airlangga; 2011 [cited 2014 27th May]. Available from: http://kuntaman-fk.web.unair.ac.id/artikel_detail-35548-Umum-Microbiology%20and%20Management%20of%20Hospital%20Infection.html.
4. Chung DR, Song JH, Kim SH, Thamlikitkul V, Huang SG, Wang H, et al. High prevalence of multidrug-resistant nonfermenters in hospital-acquired pneumonia in Asia. *American journal of respiratory and critical care medicine*. 2011;184(12):1409-17.
5. Munoz-Price LS, Jacoby GA. Extended-spectrum betalactamases 2014 [cited 2014 9th August]. Available from: <http://www.uptodate.com/contents/extended-spectrum-beta-lactamases>.
6. Tsai SS, Huang JC, Chen ST, Sun JH, Wang CC, Lin SF, et al. Characteristics of *Klebsiella pneumoniae* bacteremia in community-acquired and nosocomial infections in diabetic patients. *Chang Gung medical journal*. 2010;33(5):532-9.
7. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer*. 2010;62(1):1-20.
8. Syahadat RM, Aziz SA. Pengaruh Komposisi Media Dan Fertigasi Pupuk Organik Terhadap Kandungan Bioaktif Daun Tanaman Kemuning (*Murraya paniculata* (L.) Jack) Di Pembibitan. 2012.
9. Ng MK, al e. Bioactivity studies and chemical constituents of *Murraya paniculata* (Linn) Jack. *International Food Research Journal*. 2012;19(4):1307-12.
10. Matos MJ, Vazquez-Rodriguez S, Santana L, Uriarte E, Fuentes-Edfuf C, Santos Y, et al. Looking for new targets: simple coumarins as antibacterial agents. *Medicinal chemistry*. 2012;8(6):1140-5.
11. Xiang JL. Kamuning 2013 [cited 2014 1st Jun]. Available from: <http://stuartxchange.com/Kamuning.html>.
12. Rao S. extendedspectrum beta-lactamases2015.
13. Parven, RM et al. Extended-Spectrum beta-lactamase producing *Klebsiella pneumoniae* from blood cultures in Puducherry, India. *Indian Journal of Medical Research*. 2011;134(3)392-395 Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clinical microbiology reviews*. 2005;18(4):657-86.
14. Zeng, Ximin & Lin, Jun. Beta-Lactamase Induction and Cell Wall Metabolism in Gram-Negative Bacteria. *Frontiers in Microbiology*. 2013;4:128
15. Iswantini D, al e. Zingiber cassumunar, *Guazuma ulmifolia*, and *Murraya paniculata* Extracts as Antiobesity: In Vitro Inhibitory Effect on Pancreatic Lipase Activity. *Hayati Journal of Biosciences*. 2011;18(1):6-10.
16. Parwata IM, Oka A, Dewi PFS. Isolasi dan Uji Aktivitas Antibakteri Minyak Atsiri dari Rimpang Lengkuas (*Alpinia galanga* L.). *Jurnal Kimia*. 2008;2(2):100-4.
17. Rahayu MD. Uji Efektivitas Antibakteri Ekstrak Lengkuas Merah (*Alpinia purpurata* K. Schum) terhadap Bakteri *Escherichia coli* secara In Vitro 2012.
18. Juliantina FR, Citra DA, Nirwani B, Nyrmasitoh T, Bowo ET. Manfaat Sirih Merah (*Piper crocatum*) sebagai Agent Anti bakterial terhadap Bakteri Gram Positif dan Bakteri Gram Negatif. *Jurnal Kedokteran dan Kesehatan Indonesia*. 2009;10-9.
19. Jasmine R, Selvakumar BN, Daisy P. Investigating The Mechanism of Action of Terpenoids and The Effect of Interfering Substances on An Indian Medicinal Plant Extracr Demonstrating Antibacterial Activity. *International Journal of Pharmaceutical Studies and Research*. 2011;2:19-24
20. Maatalah BM et al. Antimicrobial activity of the alkaloids and saponin extracts of *anabasis articulata*. *Journal of Biotechnology and Pharmaceutical Reserarch*. 2012;3(3):54-57.
21. Biswas D, Roymon MG. Validation of antibacterial activity of saponin against diarrheagenic *E.coli* isolated from leaves and bark of *Acacia arabica*. *Journal of Phytochemistry*. 2012;4:21-23
22. Hasan SM, Byrd JA, Cartwright AI, Bailey CA. Hemolytic and antimicrobial activites differ among saponin-rich extract from guar, quillaja, yucca, and soybean. *Applied Biochemistry and Biotechnology*. 2010;162:1008-1017.
23. Omojate Godstime C, Enwa Felix O, Jewo Augustina O, Eze Christopher O. Mechanisms of Antimicrobial Actions of Phytochemicals Against Enteris Pathogens-A Review. *Journal of Pharmaceutical, Chemical and Biological Sciences*. 2014;2(2):77-85
24. Gautam MK, al e. In-vitro antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata* Linn. Leaves. *Asian Pacific journal of tropical biomedicine*. 2012:1660-3.
25. Sundaram M, al e. Studies on in vitro Antibacterial, Antifungal Property and Antioxidant Potency of *Murraya paniculata*. *Pakistan Journal of Nutrition*. 2011;10(10):925-9.
26. Zhou XZ, Jia F, Liu XM, Yang C, Zhao L, Wang YJ. Total alkaloids from *Sophora alopecuroides* L. increase susceptibility of extended-spectrum beta-lactamases producing *Escherichia coli* isolates to cefotaxime and ceftazidime. *Chinese journal of integrative medicine*. 2013;19(12):945-52.
27. Mahdi Askari, Amirhossein Sahebkar Mehrdad Iranshahi. Synthesis and Purificawtion of 7-Prenyloxy coumarins and Herniarin as Bioactive Natural Coumarins. *Iranian Journal of Basic Medical Sciences*. 2009;12(2):63-69
28. Safdari H, Neshani A, Sadeghian A, Ebrahimi M, Iranshahi M, Sadeghian H. Potent and selective inhibitors of class A beta-lactamase: 7-prenyloxy coumarins. *The Journal of antibiotics*. 2014;67(5):373-7.
29. Orhan DD, Ozcelik B, Ozgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological research*. 2010;165(6):496-504.

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Review Article

COVID-19 and Endothelial Dysfunction: Biomarkers and Potential Drug Mechanisms

Andrianto¹, Ronaldi Rizkiawan¹, Primasitha Maharany Harsoyo¹, Syafira Yasmine²

¹ Department of Cardiology and Vascular Medicine, School of Medicine, Universitas Airlangga - Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

² School of Medicine, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

Since the first report of pneumonia outbreak in Wuhan by the end of 2019, Coronavirus Disease 2019 (COVID-19) has become a global pandemic; causing millions of deaths globally and affecting the rest of worldwide population. The disease is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which enters hosts by inhabiting Angiotensin-Converting Enzyme-2 (ACE-2) receptors expressed in the endothelium layer of not only the respiratory tracts, but also various organs in the body. COVID-19 has been reported to trigger multiple cardiovascular manifestations. Since endothelial dysfunction plays an important role in cardiovascular events and the endothelium is heavily involved in COVID-19 pathophysiology, it is important to investigate their associations and previously established drug potencies to improve endothelial functions as possible treatment options for COVID-19. In this review, we summarize endothelial dysfunction biomarkers involved in COVID-19 and drugs that have shown potential endothelial protective properties to better understand the incidence of endothelial dysfunction in COVID-19 and its future treatment. We searched in PubMed, Wiley Online Library, EBSCO, ScienceDirect databases for literatures containing following keywords: "Endothelial dysfunction", "COVID-19", and "biomarkers". Eligible publications were then assessed and studied to comprise our literature review. A total of 96 studies matched our criteria and provided scientific evidences for our review. Materials were then compiled into a review summarizing endothelial biomarkers involved in COVID-19 and potentially repurposed drugs targeting endothelium for COVID-19. Various endothelial dysfunction biomarkers were found to be elevated in COVID-19 and is found to be related to its severity, such as adhesion molecules, selectins, PAI-1, and von Willebrand Factors. Multiple drugs targeting the endothelium are also potential and some are under investigation for COVID-19.

Keywords: COVID-19, endothelial dysfunction, SARS-COV-2, biomarkers

ABSTRAK

Sejak pertama kali pelaporannya sebagai wabah pneumonia di Wuhan pada akhir 2019, Corona Virus disease 2019 (COVID-19) telah menjadi pandemi global yang menyebabkan jutaan kematian dan memengaruhi populasi di seluruh dunia. Penyakit ini disebabkan oleh SARS-CoV-2 yang menginvasi sel inang dan berikatan dengan reseptor angiotensin-converting enzyme-2 (ACE-2) yang diekspresikan lapisan endotel saluran pernapasan serta berbagai organ lain dalam tubuh. COVID-19 telah dilaporkan dapat menimbulkan berbagai manifestasi gangguan kardiovaskular. Mengingat peran penting disfungsi endotel yang berperan dalam kejadian kardiovaskular dan patofisiologi COVID-19, maka penting menyelidiki hubungan di antaranya. Selain itu, perlu dilakukan telaah mengenai potensi obat yang sebelumnya telah terbukti memperbaiki fungsi endotel sebagai pilihan terapi yang digunakan untuk COVID-19. Pada studi ini, kami memaparkan beberapa biomarka disfungsi endotel yang terlibat pada COVID-19 dan obat-obatan yang melindungi endotel sebagai terapi potensial COVID-19 di masa mendatang. Kami melakukan pencarian di database PubMed, Perpustakaan Online Wiley, EBSCO, dan ScienceDirect untuk literatur yang berisi kata kunci berikut: "Disfungsi endotel", "COVID-19", dan "biomarker". Publikasi yang memenuhi syarat kemudian dinilai dan ditelaah untuk menyusun tinjauan pustaka. Sebanyak 96 publikasi memenuhi kriteria dan memberikan

* Corresponding Author:
andrianto@fk.unair.ac.id

bukti ilmiah untuk tinjauan pustaka ini. Materi kemudian disusun menjadi ulasan yang merangkum biomarker-biomarker endotel yang terlibat dalam COVID-19 dan obat-obatan dengan target endotel yang potensial. Berbagai biomarker disfungsi endotel ditemukan meningkat pada COVID-19 dan terkait dengan tingkat keparahannya, seperti molekul adhesi, selektin, PAI-1, dan Faktor von Willebrand. Beberapa obat yang menargetkan endotel juga ditemukan memiliki potensi dengan beberapa tengah diinvestigasi lebih lanjut sebagai terapi COVID-19.

Kata kunci: COVID-19, disfungsi endotel, SARS-CoV-2, biomarka

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INTRODUCTION

In December 2019, a novel corona virus was isolated from the respiratory tract epithelial layer of a patient with pneumonia of unknown cause in Wuhan, China. By November 2020, the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), and its consequential disease, Coronavirus Disease 2019 (COVID-19), has now infected more than 46 millions of people all over the world and caused more than 1.2 million global death.¹

Beside instigating substantial pulmonary disturbances such as pneumonia and acute respiratory distress syndromes, COVID-19 is known to trigger extrapulmonary responses, including cardiovascular manifestations. Several reported cardiovascular manifestations of COVID-19 include myocardial ischemia, arrhythmia, and even cardiogenic shock.^{1,2}

Endothelial dysfunction is a significant contributor of various cardiovascular diseases mechanism. Endothelial cells play a major role in maintaining blood tissue non-thrombogenicity, regulating thrombosis, thrombolysis, thrombocyte adherence, vascular tone, and blood flow.¹ Any disturbance of endothelial function may trigger a progression of cardiovascular problems.³

SARS-CoV-2 exploits Angiotensin-Converting-Enzyme-2 (ACE-2) as a receptor to attack human cells. ACE-2, a main target component in various cardiovascular diseases mechanism and drugs, is widely expressed in both respiratory endothelial layers and vascular endothelial layers. Therefore, it is important to learn of the endothelial involvement in COVID-19 related cardiovascular function alterations as well as the changes of biomarkers it comprises.^{1,2}

DISCUSSION

SARS-CoV-2 related endothelial changes

The innermost part of the vascular system is comprised of a single layer of endothelial cells called endothelium. A healthy endothelium maintains blood fluidity through platelet reactivity regulation, coagulation, and smooth thrombolysis by synthesizing and responding to vasoactive molecules accordingly.² The endothelium, alongside its primary immunoregulatory properties, also plays an important role in maintaining dynamic interactions between pro-coagulant and fibrinolytic factors within the vascular system. In its inactive state, the endothelium acts as a barrier that separates pro-thrombotic subendothelial layers and procoagulant factors carried within the circulation.³

SARS-CoV-2 directly affects the endothelial cells due to the high expression of ACE-2 receptors and Transmembrane Protease, Serine 2 (TMPRSS2) enzyme. After being bound by SARS-CoV-2, ACE-2 receptors are internalized such that the diminishing number of ACE-2 in endothelial cells promotes inflammation and thrombosis, triggered by subsequent hyperactivity of local Angiotensin-II (Ang-II). The decrease of ACE-2 receptors also reduces a number of conversions it normally mediates, including Angiotensin 1-7 from Ang-II, which acts as a vasoactive ligand of the MAS receptor. This causes reduction of MAS receptor activation and induction of pro-inflammatory phenotypes through the increase of type-1 Angiotensin receptor (AT₁R) activation. Furthermore, the decrease of ACE-2 receptor prevents the degradation of des-Arg-9-

Bradykinin (DABK) into inactive peptides, which subsequently raises prothrombotic signals through bradykinin receptors (BKRs) activation.^{4,5}

Endothelial activation and subsequent dysfunction is marked by imbalance of endothelium-released vasomotor factors, expressions of inflammatory cytokines and chemokines, expression and secretion of selectins and adhesive molecules as well as modulation of local thrombotic pathway.⁶ The release of cytokines and pro-inflammatory chemokines by activated macrophages augments the vicious circle of vascular integrity, coagulation, and thrombosis disturbances through reduction of endothelial glycocalyx, activation of coagulation system, and dampening of anticoagulation mechanism. Endothelial cell adhesive phenotypes induced by pro-inflammatory cytokines and chemokines promote neutrophil infiltration, producing large quantity of histotoxic components such as reactive oxygen species (ROS) and Neutrophil Extracellular Traps (NETs).

Endothelial activation is the transition of static phenotype to a specific phenotype involving responses to host immunity.⁵ Endothelial cells activation causes an increase of inflammatory cytokines and adhesion molecules, triggering releases of leukocyte, adhesion, and migration

to subendothelial chambers, constituting fundamental process of atherosclerotic lesion initiation, progress, and destabilization.⁵ Activated endothelial cells begin coagulation process by expressing P-selectin, von Willebrand factor (vWf), and fibrinogen, causing massive platelet binding, formation of fibrin and clotting of Red Blood Cells (RBC), which finally results in systemic thrombosis and Disseminated Intravascular Coagulation (DIC).⁴

Measurable biomarkers of endothelial activation and dysfunction

Activated endothelial cells express increased levels of E-selectin, P-selectin, Intercellular Adhesion Molecule 1 (ICAM-1), and Vascular Cell Adhesion Molecule 1 (VCAM-1). Upregulations of E-selectins, ICAM-1, and VCAM-1 are mediated at transcription level. E-selectin induces the rolling of circulatory leukocytes. VCAM-1 and ICAM-1 induces strong adhesion by binding Very Late Antigen 4 (VLA4) and Leucocyte Function Antigen-1 (LFA-1). After strong adhesion, leukocyte migrates through endothelial cells into its underlying tissues. Among these molecules, the adhesion molecules (ICAM-1 and VCAM-1), selectins (E-selectin and P-selectins), plasminogen activator inhibitor-1 (PAI-1), and

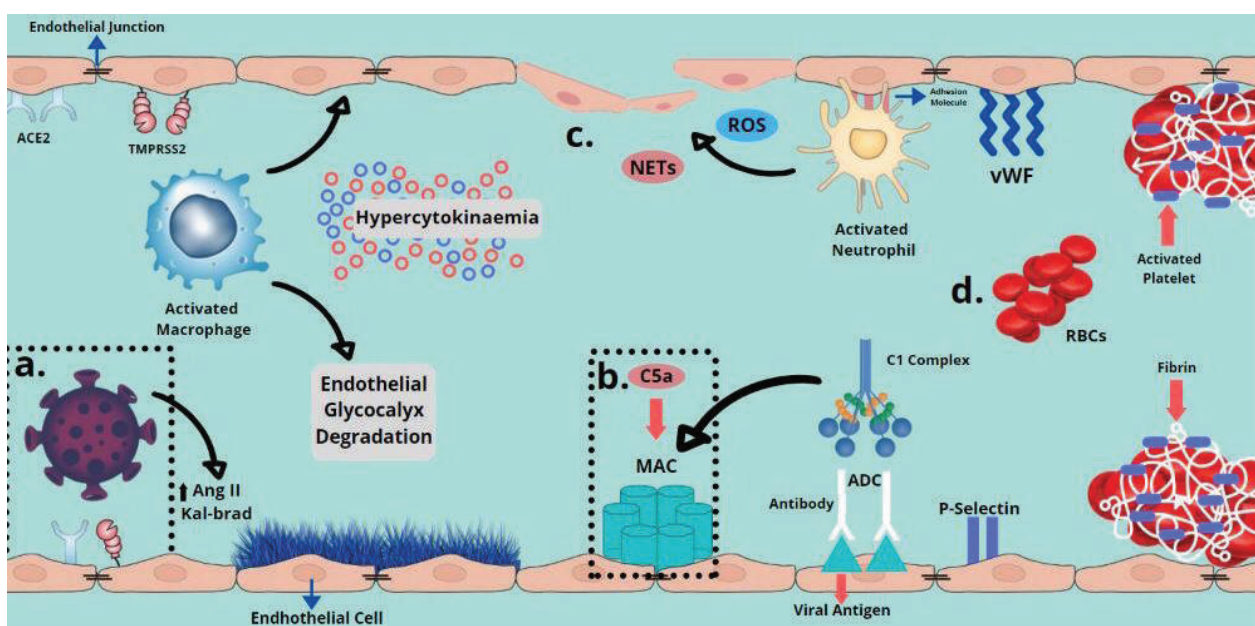


Figure 1. Alterations of endothelial, systemic coagulation and thrombotic functions due to SARS-CoV-2 infection.⁶

von Willebrand Factor (vWF) are easily obtained from the circulation and well measured by commercial immunoassays.⁵

Adhesion molecules

Endothelial dysfunction can be detected by the increase of circulating cellular adhesion molecules (CAM), including ICAM-1 and VCAM-1. ICAM-1 and VCAM-1 are minimally expressed in inactive endothelial cells. However, these expressions can be augmented by activation of cytokines and endotoxin liposaccharides. Thrombin and histamine stimulations selectively induce endothelial P-selectin expression, whereas cytokines and liposaccharides stimulations induce E-selectins expression.^{7,8}

Studies have shown that endothelial cellular adhesion markers, VCAM-1 and ICAM-1, are increased in COVID-19 patients compared to control group.⁹ These markers were also found significantly higher in severe COVID-19 infections, associating the severity of COVID-19 infection with increase of serum VCAM-1 and ICAM-1.⁹ Likewise, the recovery of severe COVID-19 infections was also associated with a decrease of serum VCAM-1 and ICAM-1 levels over time. Therefore, it could be inferred that according to the study, the increase of endothelial cellular adhesion molecule expression is correlated with the presence of COVID-19 and its severity, as well as indicating a contribution to patient's coagulopathy state.⁹ To further support this, a previous postmortem study had demonstrated a marked endothelial dysfunction in COVID-19 patients with significant increase of ICAM-1.¹⁰

Selectins

Besides VCAM-1 and ICAM-1, endothelial cells also express P-selectin and E-selectin. P-selectin is a cellular adhesion molecule stored inside the endothelium and thrombocytes which would be rapidly deployed into plasma membrane upon activation.¹¹ As infection progresses, P-selectin increases platelet aggregation and platelet-endothelium interaction. Soluble P-selectin (sP-selectin) is produced through enzymatic release of mobilized P-selectins during

inflammation.¹² Experiments with genetically engineered rat in pro-coagulant state revealed high level of sP-selectin expressions. On the other hand, sP-selectin was also proven to be a significant marker of several inflammatory and pro-coagulopathy disorders, including systemic inflammatory responses.^{11,14,15} Most recent study showed increase of sP-selectin in COVID-19 cases, further supporting the theory of COVID-19 being an endothelial dysfunction disorder.¹⁶

E-selectin can also provide as markers of endothelial dysfunction. E-selectin (CD62E) is a leukocyte adhesion molecule that is expressed by activated endothelial cells. Endothelial cells of normal skin and bone marrow or in the case of infantile hemangioma constitutively express E-selectin, in contrast with endothelial cells from other tissues that do not.^{18,19} However, these expressions are tightly regulated by inflammatory cytokines. Soluble E-selectins (sE-selectin) are released during inflammation and has long been proposed as a biomarker of endothelial dysfunction, especially in cases of sepsis.²⁰ Therefore, increased circulating sE-selectin alongside mRNA increase confirm the presence of endothelial dysfunction in COVID-19 as well as a linear correlation with its severity. Smadja *et al.* found in their study that there was an increase of E-Selectin level in COVID-19 patients. This strengthens the theory of endothelial dysfunction in COVID-19.²¹

Plasminogen activator inhibitor-1 (PAI-1)

Procoagulant condition formed by endothelial activation can also be measured from alterations of balance between tissue plasminogen activator (t-PA) and its endogen, plasminogen activator inhibitor-1.²² Plasminogen activator inhibitor-1, also known as endothelial PAI, is a serine protease inhibitor (serpin) which serves as main inhibitor of t-PA and urokinase type plasminogen activator (u-PA), a fibrinolytic agent with recent additional non-fibrinolytic properties reported.²³⁻²⁶ While also secreted by other tissues such as adipose tissue, PAI-1 is mainly produced by endothelium. Increase of PAI-1 is a risk factor of thrombosis and atherosclerosis.^{2,22}

One characteristic of COVID-19 is leukocyte sequestration, especially neutrophils, in the pulmonary microvasculature—which contributes to alveolar injury and infinite inflammation.²⁷ Local pro-inflammatory environment is exaggerated by the formation of NET which results in mass production of pro-inflammatory cytokines.²⁸ These cytokines trigger endothelial cells activations and possibly promotes release of t-PA and PAI-1.^{28,29} Activated endothelial cells expresses raised levels of PAI-1, inhibiting t-PA and u-PA, further instigating hemostasis balance alteration to procoagulant state.¹⁷

In a study by Zuo, PAI-1 level is found to be raised in COVID-19 patients.³⁰ This raise has a significant correlation between PAI-1 as well as circulating absolute neutrophil and calprotectin. This supports the presence of endothelial dysfunction in COVID-19.³⁰ Besides endothelial activation, there is a possibility that direct infection and endothelial cells destruction by SARS-CoV-2 cause a potential release of t-PA and PAI-1.³¹

A study by Kang *et al* revealed that in advanced cases of COVID-19 with severe respiratory dysfunction, the PAI-1 level is significantly higher than sepsis, acute respiratory distress syndrome (ARDS), or even burn cases.³² The significant increase of PAI-1 in severe cases of COVID-19, which are comparable to ARDS, had shown to induce vascular endothelium destruction. SARS-CoV-2 had also shown to directly infect the vascular endothelium, triggering endothelitis which indicates vascular endothelial destruction in patients with COVID-19.³¹ Collectively, this finding suggests that increased PAI-1 level promotes endotheliopathy and coagulopathy in severe cases of COVID-19.³²

Intensive care patients with severe conditions were reported to have significantly increased level of PAI-1 compared to non-intensive care patients. Data from a study by Nougier clearly demonstrated that balance between coagulation and fibrinolysis diminished in COVID-19 patients in significant hypercoagulability state related to hypofibrinolysis caused by high increase of PAI-1 level.³³ In a study by Blasi, plasma PAI-1 level

was found 3.7 times higher in COVID-19 patients compared to control.³⁴

Von Willebrand Factor

Von Willebrand Factor (vWF) is a major multidomain adhesive glycoprotein derived from endothelium, released into circulation by activated endothelial cells.^{35,36} vWF binds with platelet glycoprotein Ib α , α Ib β 3 and endothelial collagen, which activates the platelets and commences platelet aggregation.³⁵ As a carrier of blood clotting factor VIII, vWF also has an important role in clotting cascade.³⁷ vWF is synthesized by endothelial cells and megakaryocytes, which is then stored as ultra-large vWF multimers or multimers with large molecular weight within the endothelial Weibel-Palade bodies or platelet α -granules.

It is well established that pathological alterations of fibrinogen, D-dimer, vWF and P-selectin have important roles in abnormal coagulation and endothelial dysfunction. Both infection and inflammation can increase plasma vWF through activated endothelial cells.^{40,41} The majority of vWF originate from endothelial cells. Endothelial cells vWF (EC-vWF), instead of platelet vWF, critically promotes thrombus formation. In accordance, EC-vWF contributes in vWF-dependent atherogenesis by raising platelet adhesion and vascular inflammation.³⁹ vWF is also a biomarker that is relatively easy to measure.⁴²

In healthy individuals, ACE2 transforms angiotensin-II into angiotensin 1-7, which stimulates endothelial cells to produce nitric oxide (NO). NO aids blood vessels in vasodilation and suppressing platelet aggregation. In COVID-19, SARS-CoV-2 occupies ACE2, subsequently raising Angiotensin-II levels. This further enhance vasoconstriction and reduce blood flow. In this process, vWF stored inside Weibel Palade bodies, increasing formation of blood clots.⁴⁴

Activation of EC-vWF in relation of COVID-19 is known as acute phase protein, released from endothelial cells as inflammatory response.⁴⁵ In this case, its high level indicates a disturbance of endothelial function.⁴⁶ Several studies had been

performed to determine the role of vWF in COVID-19. COVID-19 patients have a documented higher level of vWF, as shown in a study by Blasi.³⁴ An increased activity of vWF and vWF antigen (vWF:Ag) was also found to be significantly raised in a study by Helms, which revealed a well-defined endothelial inflammation with a very high level of vWF:Ag.⁴⁷ Morici *et al.*, in their study, reported a significantly higher vWF in all COVID-19 patients, supporting previous studies.⁴⁸ Katneni *et al.* and several other previous publications similarly reported that vWF level was higher in COVID-19 patients.⁴⁹⁻⁵³ In a study by Panigada, the increase of vWF was reported up to 863 U/dL, further supporting evidences of endothelial dysfunction in COVID-19 patients and its potential use as a biomarker in COVID-19.⁵⁴

Fraser, in his study, measured three thrombosis factors and five markers of endothelial cell injury from the plasma using ELISA, showing significant higher levels in intensive care COVID-19 patients compared to healthy individuals.¹⁶ A study by Rauch has also shown evidences of associations between coagulation marker level on admission, amongst which are factor VIII and vWF, with the severity of COVID-19.⁵⁵ In other words, increased levels of vWF in COVID-19 patients is a potential biomarker associated with severity

of the disease. Previous studies have shown the ability of pulmonary virus to promote platelet-endothelium interactions through upregulation of endothelial ICAM-1, vWF, and fibronectins, culminating ongoing pulmonary injury.⁵⁶

State of hypoxia also triggers vWF expression and its following detachment from endothelial Weibel-Palade bodies.^{57,58} VWF upregulation induced by hypoxia has been associated with the presence of coronary and pulmonary vessels thrombus formation, as well as promoting leukocyte recruitment.⁵⁹ Accordingly, hypoxemia state observed in COVID-19 patients induce prothrombotic condition through upregulation of t-PA inhibitor and stimulation of procoagulant endothelial synthesis, including tissue factor and vWF.^{57,60-62}

VFW is an acute phase response protein released by activated endothelial cells as a reaction to inflammatory stimuli. This increase in activity of vWF and its antigen level contribute to platelet aggregation. In another study, cases of COVID-19 can cause up to 490% raise of vWF and vWF:Ag level. VWF is a main determinant of platelet adhesion after vascular injury and its resulting blood clot. High vWF:Ag level is an independent risk factor for ischemic stroke and myocardial infarction.⁶⁴ Thus, thrombocyte

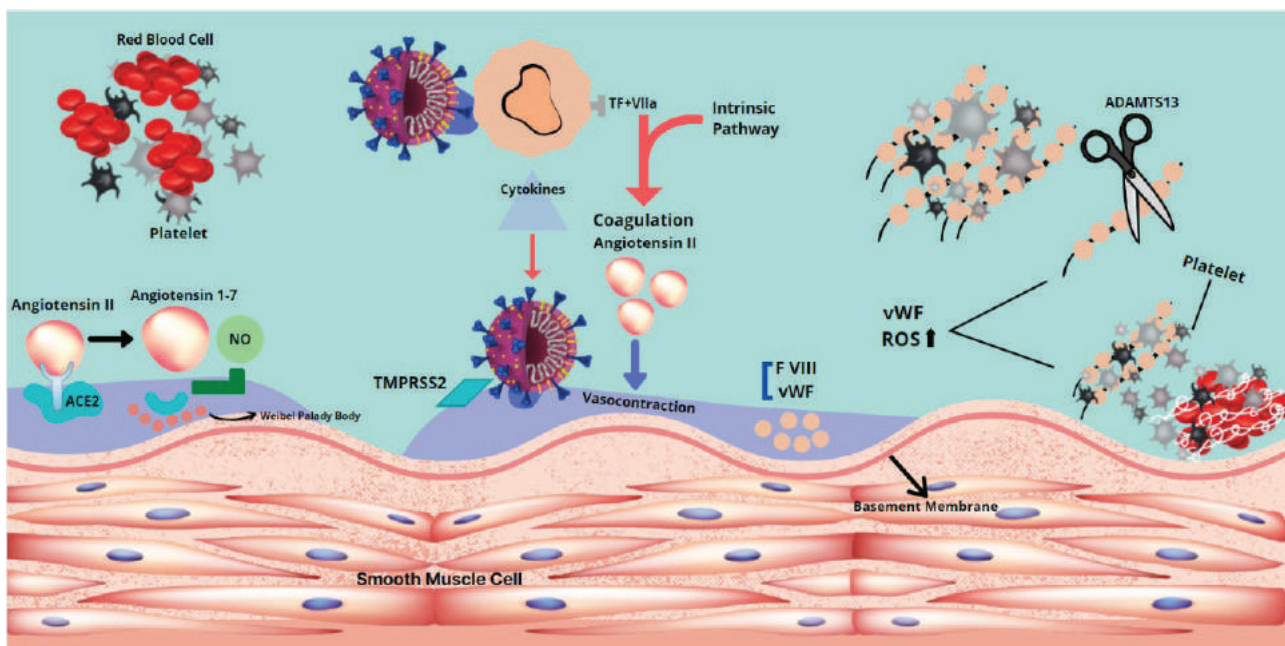


Figure 2. Thrombus formation in COVID-19.

inhibitors can be considered in prevention of cardiovascular events in COVID-19.⁶⁵

Potential mechanisms of cardiovascular drugs in COVID-19 related endothelial function repair

Various experimental and clinical studies have shown multiple currently used of investigated drugs currently can repair endothelial function, although they may have different structures and mechanisms. Among those drugs are Angiotensin Converting Enzymes (ACE) inhibitor (ACEi), Angiotensin-II Receptor Blocker (ARB), statins and antioxidants.

ACEi and ARB

Evidences have established the role of ACE inhibitors in repairing endothelial functions in animal models with heart failure and in animal models with coronary artery disease.^{66,67} ACE inhibitors elevate endothelial functions by reducing Angiotensin-II, while regulating endothelial Nitric Oxide Synthase (eNOS) and inhibiting production of reactive oxygen species (ROS), giving the drug its protective properties for endothelium.⁶⁸⁻⁷⁰ In the TREND study, quinapril, compared to placebo, repaired endothelial dysfunction in normotensive patients with coronary artery disease.⁶⁷ Administration of ARB has shown to improve endothelial function and demonstrate an overall reduction of inflammatory biomarkers, implying its importance in the pathogenesis of atherosclerosis.⁷¹ A previous meta-analysis showed that ACEi improves endothelial function in patients with endothelial dysfunction of multiple causes.⁷²

However, despite available studies mentioned, there has not been any consensus related to COVID-19 related endothelial dysfunction and ACEi or ARB as its potential clinical approach. Several available meta-analysis regarding effectivity of ACE inhibitor or ARB administration for endothelial dysfunction in COVID-19 still have differing conclusions.^{73,74} Further investigation is necessary, especially considering that ACE-2 receptor is a binding site of SARS-CoV-2.

Statins

Beneficial effects of statin in endothelial function involves multiple mechanisms. Statins improve endothelial dysfunction due to their LDL lowering properties, considering LDL and OxLDL capability in reducing eNOS.^{75,76} Statin improves NO bioactivity by activating eNOS through PI3K/Akt signaling pathway.⁸⁰ The benefits of statin for endothelial function is also related with its anti-inflammation and antioxidant properties.⁷⁷ Statins provide direct antioxidant effects to LDL through reducing LDL electronegative forms.^{78,79}

In 1995, randomized control trial stated that lovastatin can return endothelial function of a coronary artery.⁸¹ Atorvastatin was shown to reduce pro-inflammatory cytokines (TNF- α , IL-1 and IL-6), ICAM-1 and C-reactive protein (CRP) in hypercholesterolemic patients.⁸² One study showed that simvastatin produces significant reduction in endothelial dysfunction markers, inflammation, oxidative stress and endothelial apoptosis; in this study, CRP reduction seemed to have been in relation to the lipid lowering property of simvastatin.⁸³ A meta-analysis showed that statin therapy is associated with significant improvement of both coronary and peripheral endothelial functions.⁸⁴ Otherwise, statin elevate endothelial progenitor cells circulation, which contribute in the long-term effects of statin in endothelial function.⁸⁵ The combination of ACE inhibitors and statin therapy have also been demonstrating a relaxation effects in coronary vessels, which is largely dependent on endothelial function through NO production.⁸⁶

Several studies reported benefits of statins in COVID-19. Masana *et al.* in their study revealed fewer deaths reported in statin-administered group compared to the non-statin-administered group. It has also been stated that statin therapy should not be halted in hospitalized patients due to lower death rate of SARS-CoV-2 infection patients who took statins before hospitalization.⁸⁷ An observational study by Omar *et al.* also reported that, in diabetic patients with COVID-19, statin users have a 12% lower chance of death during hospitalization compared to those who did not.⁸⁸ A retrospective cohort in Singapore

linked independent use of statin with lower ICU admission rate.⁸⁸ Evidence by Sophia *et al* suggested beneficial use of continuous statin for hospitalized COVID-19 patients, as it correlates with lower chance of invasive mechanical ventilation support.⁸⁹ However, there has not been a randomized controlled trial which further supports the use of statins in COVID-19.

Antioxidant

Several substances such as vitamin C, vitamin E and N-acetylcysteine provide an antioxidant effect through different mechanisms. Vitamin C protects the endothelium by eliminating superoxide, which in turn prevents NO decomposition, lipid peroxidation, platelet and neutrophil activation, as well as upregulation of adhesion molecules.^{90,91} Vitamin C scavenges reactive nitrogen species yielded by peroxidase and inhibits myeloperoxidase/H₂O₂.⁹² Vitamin E acts as lipid soluble antioxidant, clearing radical hydroperoxyl in lipid environment.⁹³ Meanwhile, the effects of N-acetylcysteine in endothelial dysfunction are related to inhibitions of NADPH oxidase expression, leukocyte adhesion and inflammatory cytokine secretion.⁹⁴ N-acetylcysteine also prevents platelet aggregation, which largely depends on vWF and collagen binding in human plasma, and inhibits upregulation of Caveolin-1 as well as strengthening endothelial barrier function in rats.⁹⁵ Further investigations may provide useful information regarding antioxidants in the management of endothelial dysfunction related to COVID-19.

CONCLUSION

SARS-CoV-2 infection, which has caused a global pandemic, involved various clinical manifestations and underlying mechanisms. This virus invades hosts by occupying ACE-2 receptors in endothelium, which signify the important role of endothelium in this disease. Available studies have demonstrated the increase of several endothelial biomarkers such as ICAM-1, VCAM-1, E-Selectin, P-Selectin, PAI-1 and vWF in COVID-19 patients, which

supports the presence of endothelial dysfunction in COVID-19, and could further provide helpful information for the detection of endothelial dysfunction in COVID-19. These findings may also explain the higher chance of individuals with comorbidity to contract COVID-19, with increased severity. Further studies investigating NO levels in COVID-19 is necessary to confirm COVID-19 related endothelial dysfunction.

The use of drugs that has been established to improve endothelial functions may be useful as a baseline therapy in COVID-19. It has also become a point of interest whether a cured COVID-19 patient has an increased risk of cardiovascular diseases in the future due to their endothelial impairment. This topic is potential for future research, and may provide helpful insight in prevention of cardiovascular system events after COVID-19.

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CONFLICT OF INTEREST

None of the authors had a conflict of interest in this study.

REFERENCES

1. Galley HF, Webster NR. Physiology of the endothelium. *Br J Anaesth* [Internet]. 2004;93(1):105–13. Available from: <http://dx.doi.org/10.1093/bja/ae163>

2. Wang M, Hao H, Leeper NJ, Zhu L. Thrombotic Regulation From the Endothelial Cell Perspectives. *Arterioscler Thromb Vasc Biol.* 2018;38(6):e90–5.
3. Siddiqi HK, Libby P, Ridker PM. COVID-19 – A vascular disease. *Trends Cardiovasc Med.* 2020;(January).
4. Perico L, Benigni A, Casiraghi F, Ng LFP, Renia L, Remuzzi G. Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. *Nat Rev Nephrol* [Internet]. 2020; Available from: <http://dx.doi.org/10.1038/s41581-020-00357-4>
5. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: Testing and clinical relevance. *Circulation.* 2007;115(10):1285–95.
6. Halcox JPJ. Endothelial Dysfunction. *Prim Auton Nerv Syst.* 2012;319–24.
7. McLean RC, Blumenthal RS. Inflammatory markers and the risk of coronary heart disease. *Cardiol Rev.* 2005;22(6):41–2.
8. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. *Circulation.* 2004;109(25):6–19.
9. Tong M, Jiang Y, Xia D, Xiong Y, Zheng Q, Chen F, et al. Elevated Expression of Serum Endothelial Cell Adhesion Molecules in COVID-19 Patients. *J Infect Dis.* 2020;222(6):894–8.
10. Nagashima S, Mendes MC, Camargo Martins AP, Borges NH, Godoy TM, Miggiolaro AFRDS, et al. Endothelial dysfunction and thrombosis in patients with COVID-19 - Brief report. *Arterioscler Thromb Vasc Biol.* 2020;(October):2404–7.
11. Furie B. P-selectin and blood coagulation: It's not only about inflammation any more. *Arterioscler Thromb Vasc Biol.* 2005;25(5):877–8.
12. Ishiwata N, Takio K, Katayama M, Watanabe K, Titani K, Ikeda Y, et al. Alternatively spliced isoform of P-selectin is present in vivo as a soluble molecule. *J Biol Chem.* 1994;269(38):23708–15.
13. André P, Hartwell D, Hrachovinová I, Saffaripour S, Wagner DD. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A.* 2000;97(25):13835–40.
14. Schrijver IT, Kemperman H, Roest M, Kesecioglu J, de Lange DW. Soluble P-selectin as a biomarker for infection and survival in patients with a systemic inflammatory response syndrome on the intensive care unit. *Biomark Insights.* 2017;12.
15. Katayama M, Handa M, Araki Y, Ambo H, Kawai Y, Watanabe K, et al. Soluble P-selectin is present in normal circulation and its plasma level is elevated in patients with thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. *Br J Haematol.* 1993;84(4):702–10.
16. Fraser DD, Patterson EK, Slessarev M, Gill SE, Martin C, Daley M, et al. Endothelial Injury and Glycocalyx Degradation in Critically Ill Coronavirus Disease 2019 Patients: Implications for Microvascular Platelet Aggregation. *Crit Care Explor.* 2020;2(9):e0194.
17. Shapiro NI, Schuetz P, Yano K, Sorasaki M, Parikh SM, Jones AE, et al. The association of endothelial cell signaling, severity of illness, and organ dysfunction in sepsis. *Crit Care.* 2010;14(5).
18. Silva M, Videira PA, Sackstein R. E-selectin ligands in the human mononuclear phagocyte system: Implications for infection, inflammation, and immunotherapy. *Front Immunol.* 2018;8(Jan).
19. Smadja DM, Mulliken JB, Bischoff J. E-selectin mediates stem cell adhesion and formation of blood vessels in a murine model of infantile hemangioma. *Am J Pathol* [Internet]. 2012;181(6):2239–47. Available from: <http://dx.doi.org/10.1016/j.ajpath.2012.08.030>
20. Cummings CJ, Sessler CN, Beall LD, Fisher BJ, Best AM, Fowler AA. Soluble E-selectin levels in sepsis and critical illness: Correlation with infection and hemodynamic dysfunction. *Am J Respir Crit Care Med.* 1997;156(2 Pt 1):431–7.
21. Smadja DM, Guerin CL, Chocron R, Yatim N, Boussier J, Gendron N, et al. Angiotensin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients. *Angiogenesis* [Internet]. 2020;23(4):611–20. Available from: <https://doi.org/10.1007/s10456-020-09730-0>
22. Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost.* 2005;3(8):1879–83.
23. Fay WP, Korthuis RJ. No Sweetie Pie: Newly Uncovered Role for PAI-1 in Inflammatory Responses to Ischemia/Reperfusion. *Physiol Behav.* 2019;176(3):139–48.
24. Praetner M, Zuchtriegel G, Holzer M, Uhl B, Schaubächer J, Mittmann L, et al. Plasminogen Activator Inhibitor-1 Promotes Neutrophil Infiltration and Tissue Injury on Ischemia-Reperfusion. *Arterioscler Thromb Vasc Biol.* 2018;38(4):829–42.
25. Vaughan DE, Rai R, Khan SS, Eren M, Ghosh AK. Plasminogen Activator Inhibitor-1 Is a Marker and a Mediator of Senescence. *Arterioscler Thromb Vasc Biol.* 2017;37(8):1446–52.
26. Ji Y, Weng Z, Fish P, Goyal N, Luo M, Myears SP, et al. Pharmacological Targeting of Plasminogen Activator Inhibitor-1 Decreases Vascular Smooth Muscle Cell Migration and Neointima Formation. *Physiol Behav* [Internet]. 2017;176(10):139–48. Available from: [file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentar na qualificação/The impact of birth weight on cardiovascular disease risk in the.pdf](file:///C:/Users/Carla%20Carolina/Desktop/Artigos%20para%20acrescentar%20na%20qualifica%C3%A7%C3%A3o/The%20impact%20of%20birth%20weight%20on%20cardiovascular%20disease%20risk%20in%20the.pdf)
27. Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. *J Thromb Haemost.* 2020;18(7):1548–55.
28. Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Heide RS Vander. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med* 2020;(January):19–21.

29. Meltzer ME, Lisman T, De Groot PG, Meijers JCM, Le Cessie S, Doggen CJM, et al. Venous thrombosis risk associated with plasma hypofibrinolysis is explained by elevated plasma levels of TAFI and PAI-1. *Blood*. 2010;116(1):113–21.
30. Zuo Y, Warnock M, Harbaugh A, Yalavarthi S, Gockman K, Lawrence DA. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. *Sci Rep*. 2020;1–13.
31. Varga Z, Flammer A, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endothelitis in COVID-19. *Ann Oncol*. 2020;(January).
32. Kang S, Tanaka T, Inoue H, Ono C, Hashimoto S, Kioi Y, et al. IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial cells in cytokine release syndrome. *Proc Natl Acad Sci U S A*. 2020;117(36):22351–6.
33. Nougier C, Benoit R, Simon M, Desmurs-Clavel H, Marcotte G, Argaud L, et al. Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. *J Thromb Haemost*. 2020;18(9):2215–9.
34. Blasi A, von Meijenfeldt FA, Adelmeijer J, Calvo A, Ibañez C, Perdomo J, et al. In vitro hypercoagulability and ongoing in vivo activation of coagulation and fibrinolysis in COVID-19 patients on anticoagulation. *J Thromb Haemost*. 2020;18(10):2646–53.
35. Löf A, Müller JP, Brehm MA. A biophysical view on von Willebrand factor activation. *J Cell Physiol*. 2018;233(2):799–810.
36. Da Silva ML, Cutler DF. Von Willebrand factor multimerization and the polarity of secretory pathways in endothelial cells. *Blood*. 2016;128(2):277–85.
37. Butera D, Passam F, Ju L, Cook KM, Woon H, Aponte-Santamaría C, et al. Autoregulation of von Willebrand factor function by a disulfide bond switch. *Sci Adv*. 2018;4(2).
38. Verhenne S, Denorme F, Libbrecht S, Vandembulcke A, Pareyn I, Deckmyn H, et al. Platelet-derived VWF is not essential for normal thrombosis and hemostasis but fosters ischemic stroke injury in mice. *Blood*. 2015;126(14):1715–22.
39. Doddappattar P, Dhanesha N, Chorawala MR, Tinsman C, Jain M, Nayak MK, et al. Endothelial cell-derived von Willebrand factor, but not platelet-derived, promotes atherosclerosis in Apoe-deficient mice. 2019;38(3):319–35.
40. Kayal S, Jaïs JP, Aguiñi N, Chaudière J, Labrousse J. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. *Am J Respir Crit Care Med*. 1998;157(3 PART I):776–84.
41. Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: The impact of cardiovascular risk factors. *Pediatrics*. 2006;117(5):1560–7.
42. Mannucci PM. von Willebrand Factor A Marker of Endothelial Damage? Greece Rome.?? *Arterioscler Thromb Vasc Biol*. 1967;14(2):188–98.
43. Grobler C, Maphumulo SC, Grobbelaar LM, Bredenkamp JC, Laubscher GJ, Lourens PJ, et al. Covid-19: The rollercoaster of fibrin(ogen), d-dimer, von willebrand factor, p-selectin and their interactions with endothelial cells, platelets and erythrocytes. *Int J Mol Sci*. 2020;21(14):1–25.
44. Iba T, Levy JH, Connors JM, Warkentin TE, Thachil J, Levi M. The unique characteristics of COVID-19 coagulopathy. *Crit Care*. 2020;24(1):4–11.
45. Kawecki C, Lenting PJ, Denis C V. von Willebrand factor and inflammation. *J Thromb Haemost*. 2017;15(7):1285–94.
46. Breakey N, Escher R. D-dimer and mortality in COVID-19: A self-fulfilling prophecy or a pathophysiological clue? *Swiss Med Wkly*. 2020;150(21–22):1–7.
47. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med* [Internet]. 2020;46(6):1089–98. Available from: <https://doi.org/10.1007/s00134-020-06062-x>
48. Morici N, Bottiroli M, Fumagalli R, Marini C, Cattaneo M. Role of von Willebrand Factor and ADAMTS-13 in the Pathogenesis of Thrombi in SARS-CoV-2 Infection: Time to Rethink. *Thromb Haemost*. 2020;120(9):1339–41.
49. Bazzan M, Montaruli B, Sciascia S, Cosseddu D, Norbiato C, Roccatello D. Low ADAMTS 13 plasma levels are predictors of mortality in COVID-19 patients. *Intern Emerg Med* [Internet]. 2020;15(5):861–3. Available from: <https://doi.org/10.1007/s11739-020-02394-0>
50. Huisman A, Beun R, Sikma M, Westerink J, Kusadasi N. Involvement of ADAMTS13 and von Willebrand factor in thromboembolic events in patients infected with SARS-CoV-2. *Int J Lab Hematol*. 2020;42(5):e211–2.
51. Adam E, Zacharowski K, Miesbach W. A comprehensive assessment of the coagulation profile in critically ill COVID-19 patients. *Thromb Res*. 2020;(January).
52. Latimer G, Corriveau C, DeBiasi RL, Jantusch B, Delaney M, Jacquot C, et al. Cardiac dysfunction and thrombocytopenia-associated multiple organ failure inflammation phenotype in a severe paediatric case of COVID-19. *Lancet Child Adolesc Health* 2020;(January):19–21.
53. Katneni UK, Alexaki A, Hunt RC, Schiller T, Dicuccio M, Buehler PW, et al. Coagulopathy and thrombosis as a result of severe COVID-19 infection: A microvascular focus. *Thromb Haemost*. 2020;
54. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, et al.

- Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost*. 2020;18(7):1738–42.
55. Rauch A, Labreuche J, Lassalle F, Goutay J, Caplan M, Charbonnier L, et al. Coagulation biomarkers are independent predictors of increased oxygen requirements in COVID-19. *J Thromb Haemost*. 2020;(August):1–12.
 56. Sugiyama MG, Gamage A, Zyla R, Armstrong SM, Advani S, Advani A, et al. Influenza Virus Infection Induces Platelet-Endothelial Adhesion Which Contributes to Lung Injury. *J Virol*. 2016;90(4):1812–23.
 57. Mojiri A, Nakhai-Nejad M, Phan WL, Kulak S, Radziwon-Balicka A, Jurasz P, et al. Hypoxia results in upregulation and de novo activation of von willebrand factor expression in lung endothelial cells. *Arterioscler Thromb Vasc Biol*. 2013;33(6):1329–38.
 58. Pinsky DJ, Naka Y, Liao H, Oz MC, Wagner DD, Mayadas TN, et al. Hypoxia-induced exocytosis of endothelial cell weibel-palade bodies: A mechanism for rapid neutrophil recruitment after cardiac preservation. *J Clin Invest*. 1996;97(2):493–500.
 59. Mojiri A, Alavi P, Lorenzana Carrillo MA, Nakhaei-Nejad M, Sergi CM, Thebaud B, et al. Endothelial cells of different organs exhibit heterogeneity in von Willebrand factor expression in response to hypoxia. *Atherosclerosis* [Internet]. 2019;282(June 2018):1–10. Available from: <https://doi.org/10.1016/j.atherosclerosis.2019.01.002>
 60. Matsuura Y, Yamashita A, Iwakiri T, Sugita C, Okuyama N, Kitamura K, et al. Vascular wall hypoxia promotes arterial thrombus formation via augmentation of vascular thrombogenicity. *Thromb Haemost*. 2015;114(1):158–72.
 61. Ogawa S, Clauss M, Kuwabara K, Shreeniwas R, Butura C, Koga S, et al. Hypoxia induces endothelial cell synthesis of membrane-associated proteins. *Proc Natl Acad Sci U S A*. 1991;88(21):9897–901.
 62. Fearn C, Loskutoff DJ. Induction of plasminogen activator inhibitor 1 gene expression in murine liver by lipopolysaccharide: Cellular localization and role of endogenous tumor necrosis factor- α . *Am J Pathol*. 1997;150(2):579–90.
 63. Gragnano F, Sperlongano S, Golia E, Natale F, Bianchi R, Crisci M, et al. The Role of von Willebrand Factor in Vascular Inflammation: From Pathogenesis to Targeted Therapy. *Mediators Inflamm*. 2017;2017.
 64. Andersson HM, Siegerink B, Luken BM, Crawley JTB, Algra A, Lane DA, et al. High VWF, low ADAMTS13, and oral contraceptives increase the risk of ischemic stroke and myocardial infarction in young women. *Blood*. 2012;119(6):1555–60.
 65. Hoechter DJ, Becker-pennrich A, Langrehr J, Bruegel M, Zwissler B, Schaefer S. Higher procoagulatory potential but lower DIC score in COVID-19 ARDS patients compared to non-COVID-19 ARDS patients. *Thromb Res* 2020;(January).
 66. Varin R, Mulder P, Tamion F, Richard V, Henry JP, Lallemand F, et al. Improvement of endothelial function by chronic angiotensin-converting enzyme inhibition in heart failure: Role of nitric oxide, prostanoids, oxidant stress, and bradykinin. *Circulation*. 2000;102(3):351–6.
 67. Mancini GBJ, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: The TREND (Trial on Reversing ENdothelial Dysfunction) study. *Circulation*. 1996;94(3):258–65.
 68. Fujii M, Wada A, Tsutamoto T, Ohnishi M, Isono T, Kinoshita M. Bradykinin improves left ventricular diastolic function under long-term angiotensin-converting enzyme inhibition in heart failure. *Hypertension*. 2002;39(5):952–7.
 69. Bachetti T, Comini L, Pasini E, Cargnoni A, Curello S, Ferrari R. ACE-inhibition with quinapril modulates the nitric oxide pathway in normotensive rats. *J Mol Cell Cardiol*. 2001;33(3):395–403.
 70. Mukai Y, Shimokawa H, Higashi M, Morikawa K, Matoba T, Hiroki J, et al. Inhibition of renin-angiotensin system ameliorates endothelial dysfunction associated with aging in rats. *Arterioscler Thromb Vasc Biol*. 2002;22(9):1445–50.
 71. Sola S, Mir MQS, Cheema FA, Khan-Merchant N, Menon RG, Parthasarathy S, et al. Irbesartan and lipoic acid improve endothelial function and reduce markers of inflammation in the metabolic syndrome: Results of the Irbesartan and Lipoic Acid in Endothelial Dysfunction (ISLAND) study. *Circulation*. 2005;111(3):343–8.
 72. Shahin Y, Khan JA, Samuel N, Chetter I. Angiotensin converting enzyme inhibitors effect on endothelial dysfunction: A meta-analysis of randomised controlled trials. *Atherosclerosis* [Internet]. 2011;216(1):7–16. Available from: <http://dx.doi.org/10.1016/j.atherosclerosis.2011.02.044>
 73. Hasan SS, Kow CS, Hadi MA, Zaidi STR, Merchant HA. Mortality and Disease Severity Among COVID-19 Patients Receiving Renin-Angiotensin System Inhibitors: A Systematic Review and Meta-analysis. *Am J Cardiovasc Drugs* [Internet]. 2020;20(6):571–90. Available from: <https://doi.org/10.1007/s40256-020-00439-5>
 74. Liu X, Long C, Xiong Q, Chen C, Ma J, Su Y, et al. Association of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers with risk of COVID-19, inflammation level, severity, and death in patients with COVID-19: A rapid systematic review and meta-analysis. *Clin Cardiol*. 2020;
 75. Martínez-gonzález J, Raposo B, Rodríguez C, Badimon L. Downregulation by Atherogenic Levels of Native LDLs Balance Between Transcriptional and

- Posttranscriptional Regulation. *Arterioscler Thromb Vasc Biol.* 2001;804–9.
76. Laufs U, Fata V La, Plutzky J, Liao JK. Upregulation of Endothelial Nitric Oxide Synthase by HMG CoA Reductase Inhibitors. *Circulation.* Ulrich. 1998;1129–35.
77. Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering - Are they clinically relevant? *Eur Heart J.* 2003;24(3):225–48.
78. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S. Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: Antiatherogenic effects of HMG-CoA reductase inhibitor therapy. *J Cardiovasc Pharmacol.* 1998;31(1):39–45.
79. Sánchez-Quesada JL, Otal-Entraigas C, Franco M, Jorba O, González-Sastre F, Blanco-Vaca F, et al. Effect of simvastatin treatment on the electronegative low-density lipoprotein present in patients with heterozygous familial hypercholesterolemia. *Am J Cardiol.* 1999;84(6):655–9.
80. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, David J, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. 2010;6(9):1004–10.
81. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The Effect of Cholesterol-Lowering and Antioxidant Therapy on Endothelium-Dependent Coronary Vasomotion. *J Occup Environ Med.* 1996;38(5):468.
82. Ascer E, Bertolami MC, Venturini ML, Buccheri V, Souza J, Nicolau JC, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis.* 2004;177(1):161–6.
83. Kirmizis D, Papagianni A, Dogrammatzi F, Skoura L, Belechri AM, Alexopoulos E, et al. Effects of simvastatin on markers of inflammation, oxidative stress and endothelial cell apoptosis in patients on chronic hemodialysis. *J Atheroscler Thromb.* 2010;17(12):1256–65.
84. Reriani MK, Dunlay SM, Gupta B, West CP, Rihal CS, Lerman LO, et al. Effects of statins on coronary and peripheral endothelial function in humans: A systematic review and metaanalysis of randomized controlled trials. *Eur J Cardiovasc Prev Rehabil.* 2011;18(5):704–16.
85. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, et al. Increase endothelial progenitor cells via the PI 3-kinase / Akt pathway. *J Clin Invest.* 2001;108(3):365–6.
86. Tiefenbacher CP, Friedrich S, Bleeke T, Vahl C, Chen X, Niroomand F. ACE inhibitors and statins acutely improve endothelial dysfunction of human coronary arterioles. *Am J Physiol - Hear Circ Physiol.* 2004;286(4 55-4):1425–32.
87. Masana L, Correig E, Borjabad CR, Anoro E, Arroyo JA, Al E. Effect of Statin Therapy on SARS-CoV-2 Infection-related Mortality in Hospitalized Patients. *Akrab Juara* [Internet]. 2020;5(1):43–54. Available from: <http://www.akrabjuara.com/index.php/akrabjuara/article/view/919>
88. Saeed O, Castagna F, Agalliu I, Xue X, Patel SR, Rochlani Y, et al. Statin Use and In-Hospital Mortality in Diabetics with COVID-19. *J Am Heart Assoc.* 2020;15(24): 1-12
89. Song SL, Hays SB, Panton CE, Mylona EK, Kalligeros M, Shehadeh F, et al. Statin use is associated with decreased risk of invasive mechanical ventilation in COVID-19 patients: A preliminary study. *Pathogens.* 2020;9(9):1–9.
90. Heitzer T, Just H, Münzel T. Antioxidant Vitamin C Improves Endothelial Dysfunction in Chronic Smokers. *Circulation.* 1996;94(1):6–9.
91. Matsumoto T, D'Uscio L V, Eguchi D, Akiyama M, Smith LA, Katusic ZS. Protective effect of chronic vitamin C treatment on endothelial function of apolipoprotein E-deficient mouse carotid artery. *J Pharmacol Exp Ther.* 2003;306(1):103–8.
92. Carr AC, McCall MR, Frei B. Oxidation of LDL by myeloperoxidase and reactive nitrogen species: Reaction pathways and antioxidant protection. *Arterioscler Thromb Vasc Biol.* 2000;20(7):1716–23.
93. Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radic Biol Med.* 2011;51(5):1000–13.
94. Scioli MG, Bielli A, Agostinelli S, Tarquini C, Arcuri G, Ferlosio A, et al. Antioxidant treatment prevents serum deprivation- and TNF- α -induced endothelial dysfunction through the inhibition of NADPH oxidase 4 and the restoration of β -oxidation. *J Vasc Res.* 2014;51(5):327–37.
95. Chen J, Reheman A, Gushiken FC, Nolasco L, Fu X, Moake JL, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest.* 2011;121(2):593–603.
96. Shapiro, N.I., Schuetz, P., Yano, K. et al. The association of endothelial cell signaling, severity of illness, and organ dysfunction in sepsis. *Crit Care* 14, R182 (2010). <https://doi.org/10.1186/cc9290>

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Research Article

Antimicrobial Activities of *Laurus nobilis* Leaves Ethanol Extract on *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*.

Khawla Abdullah Sakran¹, Dadik Raharjo^{2*}, Ni Made Mertaniasih³

¹Immunology Study Program, Post Graduate School, Universitas Airlangga, Surabaya, Indonesia

²Gastroenteritis and Salmonellosis Laboratory, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

³Department of Clinical Microbiology, Faculty of Medicine Universitas Airlangga

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ABSTRACT

Laurus nobilis is one of the most well-known, most frequently used plants is from Lauraceae family which contains up 2.500 species that grow in the subtropics and tropics of the Mediterranean region and Indonesia. This study was supposed to investigate the antimicrobial effect of *L.nobilis* leaves ethanol extract on *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*. This preliminary study examined the antimicrobial effect of *L.nobilis* leaves ethanol extract. The method used Agar-well diffusion for determination of the zone of inhibition and the minimum bactericidal concentration to investigate the activity of *L.nobilis* leaves ethanol extract at 100% concentration. The results revealed that extract of *L. nobilis* leaves had the antibacterial activity against *Staphylococcus aureus* with a zone of inhibition (16.3 ± 1.5 mm), *Staphylococcus aureus* with (14.5 ± 0.5 mm), and weak antimicrobial activity against *Escherichia coli* (11.3 ± 1.1 mm). Also, through the minimum bactericidal concentration experiment, the *L.nobilis* leaves ethanol extract had activity on *Staphylococcus aureus* and *Salmonellae typhi*, it's killed the bacteria in all concentration start it from 5×10^7 to 5×10^4 . But the activity on *Escherichia coli* just weaken concentration 5×10^7 and 10^6 . This research has concluded that the *L.nobilis* leaves ethanol extract exhibited a significant antimicrobial effect against *Staphylococcus aureus* and *Salmonellae typhi* then *Escherichia coli* that is considered a kind of multidrug-resistant bacteria.

Keywords: Antibacterial Activity; *Laurus nobilis* leaves ethanol extract; *Staphylococcus aureus*; *Salmonellae typhi*; *Escherichia coli*.

ABSTRAK

Laurus nobilis has been known for a long time as a plant that is efficacious for preventing and treating several kinds of diseases. This study aims to determine the antimicrobial effect of *L. nobilis* leaves extracted using ethanol against *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*. The research method used agar diffusion to measure the zone of inhibition against the test bacteria. Gentamicin and DMSO were used as positive and negative controls. The results showed that *Laurus nobilis* extract had antibacterial activity against *Staphylococcus aureus* with an inhibitory zone (16.3 ± 1.5 mm), *Salmonellae typhi* with a zone (14.5 ± 0.5 mm), and against *Escherichia coli* (11.3 ± 1.1 mm).

Kata kunci: Antibakteri; *Laurus nobili* etanol ekstrak; *Staphylococcus aureus*; *Salmonellae Typhi*; *Escherichia coli*.

* Corresponding Author:
dadik_tdc@yahoo.co.id

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INTRODUCTION

In 2018, The WHO Global Antimicrobial Surveillance System reported that 500,000 people with suspected bacterial infections across the globe is attributed to the antibiotic resistance. *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli* are the most common human pathogens that are consistently causing different sequelae of infection in both genders and all ages.¹ These pathogens have also a significant number of morbidities and mortalities, particularly in developing countries. The bacteria develop a resistance to antimicrobials by different mechanisms whereby limiting uptake of the drug, enzymatic inactivation of the drug, modification of the drug target, and active efflux of the drug. Depending on the antimicrobial involved, the bacteria may use one or several of these resistance mechanisms.²

Pathogenic *E. coli* is resistant to various antibiotics and including the strain that extended-spectrum b-lactamase (ESBL).³ where *E. coli* considered the most pathogenic bacteria that causes of diarrhea in humans and animals.⁴ Increased resistant of the *S. typhi* to antimicrobial drugs was reported and may allow it to cross the intestinal mucosa to the bloodstream and infects deep organs such as the bones, joints, and meninges.⁵ Methicillin-resistant *S. aureus* (MRSA) is a major pathogen associated with serious community and hospital-acquired disease where these strains showed resistance to a wide range of antibiotics, thus limiting the treatment options to very few agents such as teicoplanin and vancomycin.^{6,7}

Antibiotic resistance is an internationally recognized health problem. This problem, in recent years is greatly threatening because of emergence of Multi-Drug Resistant organisms (MDRO).^{8,9} New antibacterial agents from many sources including herbal products that are preferred over

traditional medicines due to its wide biological activity, safety and lower cost. The herbal products contain groups of effective compounds that can be investigated for effectiveness as antimicrobials, antioxidants, antiseptic, and anti-inflammatory. Herbal products are increasingly used as a dietary supplement to fight against infection and lower the risk in population.^{10,11}

L. nobilis is one of the most well-known and most frequently used plants and it is member lauraceae family which contains up 2,500 species that grow in the subtropics and tropics of the Mediterranean region include Indonesia. Most species possess aromatic stems, roots, leaves, and fruits.^{12,13}

As a medicinal plant, its leaves and fruits have been known since long time ago as a species that can be used for therapy against rheumatism, skin rashes, earaches, stomachache, astringent, carminative, diaphoretic, stimulant, emetic, emmenagogue and abortifacient.¹² In addition, its Volatile oil is used by the cosmetic industry in creams, perfumes, and soaps. It has a lot of chemical properties that are useful in manufacturing of medicine, for instance, it represents a basic material in dentistry such as alkaloids, flavonols, phenolic, flavones (apigenin and luteolin), glycosylate flavonoids, cysterpine and soliterpinat to fight against or prevent common diseases.^{14,15}

Several studies described and confirmed that extracting phytochemicals and active ingredients of herbal remedies give medicinal benefits more than the use of the herb itself.¹⁶ Many studies, for example Yilmaz *et al* (2017) and Aldhafer *et al* (2017) have found that the essential oil of *L.nobilis* leaves has strong antibacterial activity against Gram negative and Gram-positive bacteria.^{17,18} Ozcan *et al.* (2016) found that the green synthesis of zinc oxide nanoparticles using the aqueous leaf extract of *L.nobilis* (Ln-ZnO NPs) were has antibacterial activity of Ln-ZnO

NPs was greater against Gram-positive (*S. aureus*) bacteria than Gram-negative (*P. aeruginosa*) bacteria.¹⁹ Therefore, the main objective of this study was to evaluate the antimicrobial activity of *L.nobilis* leaves extract against *S. aureus*, *S. typhi* and *E. coli*.

MATERIAL AND METHODS

Preparation of the *L. nobilis* leaves ethanol extract.

Fresh *L. nobilis* leaves ethanol extract (Daun Salam) weighing 5 kilograms (kgs) were collected from a farm in Malang Indonesia in October 2019. Its were washed under running tap water, air dried and finely grinded with a blender. 500g of the finely grinded leaves were then soaked in 300mls of 70% ethanol in an airtight container for 24 hours. The mixture was filtered using filter paper 11µm and the solute was extracted with a rotary evaporator at 45°C were the final volum of extract 100ml then stored in -20 °C before used.^{20,21,22}

Antimicrobial assay by agar-well diffusion method

This study was an evaluation which was intended to assess the antimicrobial activity of *L. nobilis* leaves ethanol extract to *S. aureus*, *S. typhi* and *E. coli*. This research was conducted in the Laboratory a BSL 3 Universitas Airlangga from November 2019 to December 2019. The used bacterial strains in this study are *S. aureus* (ATCC 25423), *E. coli* (ATCC 25922) and *S. typhi* (BSL 2 Lab. collection). Strains were overnight grown onto plates of Muller-Hinton agar (MHA).

Antimicrobial activity was carried out using the agar well diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI).^{23,24} Three to five colonies of each bacterium were dissolved in 2 ml of physiological saline and the turbidity was adjusted to 0.5 Mac Farland's turbidity which is equivalent to 0.5×10^8 bacteria per ml of solution. a swab use to spread the bacteria on surface of MHA media and then applied 100µl of *L.nobilis* leaves ethanol extract at concentration 100% on labeled well and 100

µl of Dimethyl Sulfoxide put on another well as a negative control and Gentamicin (10µg) disc was used as a positive control. The plate were incubated at 37°C for 24 h and the antibacterial activity determined by an inhibition zone (IZ) that formed around the well. The IZ of *L.nobilis* leaves ethanol extract was measure using calipers and compared with IZ gentamicin.²⁵

RESULTS AND DISCUSSION

Through our experiments as shown in Fig. (1) and Table 1 the results of agar well diffusion assay showed the IZ of *L.nobilis* leaves ethanol extract to *S. aureus* (16.3 ± 1.5 mm), followed by *S. typhi* (14.5 ± 0.5 mm) and *E. coli* (11.3 ± 1.1 mm). This results were shown that the *S. aureus* were most sensitive against *L.nobilis* leaves ethanol extract. This finding was in tandem with the results published by Al-Ogaili (2020) which highlighted the great inhibition activity of *L.nobilis* leaves ethanol extracts to this Gram-positive bacterium.²⁹ As reported by Otsuka et al. (2008) the *L. nobilis* has antimicrobial activity against methicillin-resistant *S. aureus* (MRSA) through purified two compound flavonoids and kaempferol, that both compounds showed strong antimicrobial activity.³²

Table 1. Antibacterial activity of *L.nobilis* leaves ethanol extracts

Microorganism	Diameter of growth of inhibition zones (mm)	
	<i>L.nobilis</i> leaves ethanol extract	Gentamicin
<i>S. aureus</i>	16.3 ± 1.5	25.6 ± 0.5
<i>S. typhi</i>	14.5 ± 0.5	20.6 ± 1.1
<i>E. coli</i>	11.3 ± 1.1	19 ± 0.5

*Values, including diameter of the well (6 mm), are means of three replicates ± SD

Figure 1. Antibacterial activity of *L.nobilis* leaves ethanol extract against bacteria (A) *Salmonella typhi* (B) *E. coli* (C) *S. aureus*.

The active compound was seen against *S. aureus*, *S. typhi* and *E. coli*. One from this Flavonoid compound has antibacterial properties because it has the capability to produce transduction energy

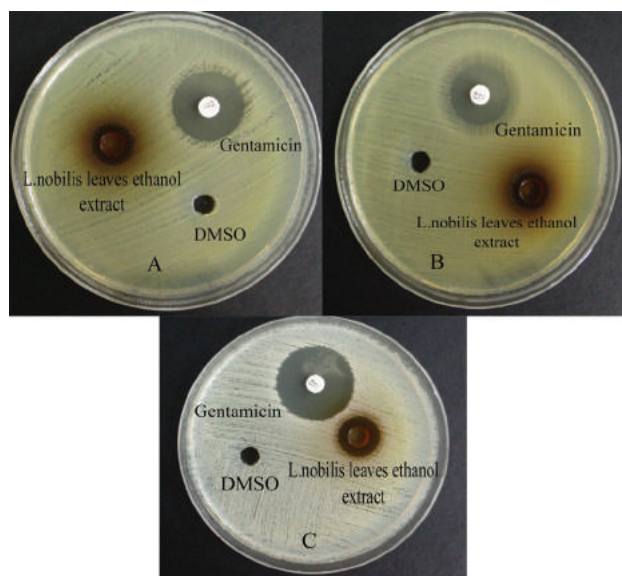


Figure 1. Antibacterial activity of *L.nobilis* leaves ethanol extract against bacteria (A) *Salmonella typhi* (B) *E. coli* (C) *S. aureus*.

that will affect the cytoplasm of the bacteria and slow down its motility, since it has an ability to interact directly with the Deoxyribonucleic acid (DNA) of the bacteria.¹⁴ Type of solvent used for extracting *L.nobilis* leaf has a major impact on their antibacterial activity. Extraction of *L.nobilis* leaf with ethanol resulted in a product with greater overall antibacterial activity. Study of Algabri that carried out on antibacterial activity of Libya bay leaf extracted with methanol and n-hexane, it was observed that the n-hexane extract showed no antibacterial activity but the methanol extract had good inhibitory activity against *S. aureus*.¹⁵ Also, El Malti and Amarouch (2009) found that the bay leaf extract has a significant antimicrobial activity against wide range of human pathogen.²⁸

Therefore, the result that we found confirmed that *L. nobilis* leaves ethanol extract has antimicrobial activity against microorganism, it's that observed the antimicrobial activity during agar well diffusion and bactericidal activity experiment. These results concurred with the result of Aldhaher that found aqueous extract had good inhibitory activity against *Streptococcus mutants* with MBCs range 30-60mg/ml. Also concurred with study of Yilmaz who found that

antimicrobial activity of the essential oil against the tested panel of food-spoiling bacteria and one yeast strain.^{14,17} Also, the study of Siriken who demonstrated that the essential oil of *L. nobilis* had strong antibacterial activity against Gram-negative and Gram-positive food-borne pathogens.²⁸ Study of Aljindan and Alkharsah, (2020) show, the resistance of *Salmonella* species to antimicrobial drugs increased from 24.6% in 2011 to 37.8% in 2018. The research study by in 2018 all *Salmonella* isolates were completely resistant to Cefalotin, Cefuroxime, and Cefoxitin, while they found some susceptibility to other Cephalosporins and Ciprofloxacin.¹⁷ While study of Patil and Mule they found *S. typhi* sensitive to Cefixime, Ceftriaxone, and Azithromycin and based on average Minimal Inhibitory Concentration and MIC breakpoints.³⁰ Through the experiment conducted on Rats by Qnais *et al* (2012) which found *L. nobilis* aqueous extract has antidiarrheal agent.³¹

Study of Nafis *et al.* exhibited notable potency regarding antimicrobial activity of (EOs) from *L. nobilis* leaves had the highest activity against *E. coli*, with MIC: 22.2 mg/mL and IZ 9.00 mm. while it had activity against *S. aureus* with IZ 10.0 mm and moderate MIC: 5.55 mg/mL.³²

CONCLUSIONS

The result of this study demonstrated an antibacterial effects of *L. nobilis* leaves ethanol extract was proved a strong antibacterial activity against bacterial infections as they exhibited an antimicrobial effect against *S. aureus*, *S. typhi* and weak effect in *E. coli*, so that is considered a kind of *drug development substance* for multidrug resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. *Frontiers in microbiology*. 2011 Sep 28;2:203.
2. Yılmaz EŞ, Aslantaş Ö. Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. *Asian Pacific journal of tropical medicine*. 2017 Nov 1;10(11):1059-64.
3. Al-Talib H, Al-Khateeb A, Hassan H. Antimicrobial resistance of *Staphylococcus aureus* isolates in Malaysian Tertiary Hospital. *International Medical Journal*. 2015 Apr 1;22(1):1-3.
4. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, Kiflay R, Tesfu T. Methicillin-resistant *Staphylococcus aureus* (MRSA): prevalence and antimicrobial sensitivity pattern among patients—a multicenter study in Asmara, Eritrea. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 2019 Jan 1;2019.
5. Alharbi NS, Khaled JM, Kadaikunnan S, Alobaidi AS, Sharafaddin AH, Alyahya SA, Almanaa TN, Alsughayier MA, Shehu MR. Prevalence of *Escherichia coli* strains resistance to antibiotics in wound infections and raw milk. *Saudi journal of biological sciences*. 2019 Nov 1;26(7):1557-62.
6. Torkan S, Bahadoranian MA, Khamesipour F, Anyanwu MU. Detection of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from diarrhoeic dogs in Iran. *Archivos de medicina veterinaria*. 2016;48(2):181-90.
7. Aljindan RY, Alkharsah KR. Pattern of increased antimicrobial resistance of *Salmonella* isolates in the Eastern Province of KSA. *Journal of Taibah University Medical Sciences*. 2020 Feb 1;15(1):48-53.
8. Bryce A, Hay AD, Lane IF, Thornton HV, Wootton M, Costelloe C. Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by *Escherichia coli* and association with routine use of antibiotics in primary care: systematic review and meta-analysis. *bmj*. 2016 Mar 15;352:i939.
9. Odonkor ST, Addo KK. Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. *International journal of microbiology*. 2018 Aug 19;2018.
10. Tambekar DH, Dahikar SB, Lahare MD. Antibacterial potentials of some herbal preparations available in India. *Res J Med Med Sci*. 2009;4:224-7.
11. Hidayat YW, Widodo AD, Dachan YP. The antimicrobial effect of+ Oxivarea against methicillin resistance *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Internet Journal of Microbiology*. 2019;16 (1):200-10.
12. Kaurinovic B, Popovic M, Vlaisavljevic S. In vitro and in vivo effects of *Laurus nobilis* L. leaf extracts. *Molecules*. 2010 May;15(5):3378-90.
13. Harismah K. Pemanfaatan Daun Salam (*Eugenia Polyantha*) Sebagai Obat Herbal Dan Rempah Penyedap Makanan. *Warta Lpm*. 2017 Feb 21;19(2):110-8.
14. Aldhaher ZA, Merza PhD WM, Almelan MF, Shaker RM, Yas LS. Effectiveness of Bay Leaves Aqueous Extract on *Streptococcus Mutans* In Comparison To Chlorhexidine Gluconate. *J Pharmacy and Biological Science*. 2017:12-6.
15. Algabri SO, Doro BM, Abadi AM, Shiba MA, Salem AH. Bay Leaves have Antimicrobial and Antioxidant Activities. *Journal of Pathogen Research*. 2018 Aug 2;1(1).
16. Klemow KM, Bartlow A, Crawford J, Kocher N, Shah J, Ritsick M. Herbal medicine: biomolecular and clinical aspects. CRC Press. 2011;2(11):211-28.
17. Yılmaz ES, Timur M, Aslim B. Antimicrobial, antioxidant activity of the essential oil of Bay Laurel from Hatay, Turkey. *Journal of Essential Oil Bearing Plants*. 2013 Feb 1;16(1):108-16. *Journal of Microbiology*. 2019 Volume 16 Number.
18. Ozcan B, Esen M, Sangun MK, Coleri A, Caliskan M. Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. *Journal of Environmental Biology*. 2010 Sep 1; 31(5): 637-41.
19. Diten POM. 1986. Sediaan Galenik. Jakarta: Departemen Kesehatan Republik Indonesia.
20. Sudjadi. 1986. Metode Pemisahan. Yogyakarta: UGM Press.
21. Nugroho, Agung. 2017. Teknologi Bahan Alam. Banjarmasin: Lambung Mangkurat University press.
22. CLSI C. Performance standards for antimicrobial susceptibility testing. *Clinical Lab Standards Institute*. 2016;35(3):16-38.
23. Balouiri M, Sadiki M, Ibsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*. 2016 Apr 1;6(2):71-9.
24. Alonso CA, Domínguez C, Heras J, Mata E, Pascual V, Torres C, Zarazaga M. Antibioqramj: A tool for analysing images from disk diffusion tests. *Computer methods and programs in biomedicine*. 2017 May 1;143:159-69.
25. Mah TF. Establishing the minimal bactericidal concentration of an antimicrobial agent for planktonic cells (MBC-P) and biofilm cells (MBC-B). *JoVE (Journal of Visualized Experiments)*. 2014 Jan 2(83):e50854.
26. Al-Ogaili N, Bilal R, Younis H, Khadim T. The examination of the water concentrates of *Laurus nobilis* leaves antibacterial activity utilizing various strategies for extraction (in vitro). *International Journal*

- of Research in Pharmaceutical Sciences. 2020 Jan 6;11(1):66-9.
27. Otsuka N, Liu MH, Shiota S, Ogawa W, Kuroda T, Hatano T, Tsuchiya T. Anti-methicillin resistant *Staphylococcus aureus* (MRSA) compounds isolated from *Laurus nobilis*. Biological and Pharmaceutical Bulletin. 2008 Sep 1;31(9):1794-7.
 28. El Malti J, Amarouch H. Antibacterial effect, histological impact and oxidative stress studies from *Laurus nobilis* extract. Journal of food quality. 2009 Apr;32(2):190-208.
 29. Sırıken B, Yavuz C, Güler A. Antibacterial Activity of *Laurus nobilis*: A review of literature. Medical Science and Discovery. 2018 Nov 30; 5(11):374-9.
 30. Patil N, Mule P. Sensitivity Pattern of *Salmonella typhi* And *Paratyphi A* Isolates to Chloramphenicol and Other Anti-Typhoid Drugs: An in Vitro Study. Infection and Drug Resistance. 2019;12:3217.
 31. Qnais EY, Abdulla FA, Kaddumi EG, Abdalla SS. Antidiarrheal activity of *Laurus nobilis* L. leaf extract in rats. Journal of medicinal food. 2012 Jan 1;15(1):51-7.
 32. Nafis A, Kasrati A, Jamali CA, Custódio L, Vitalini S, Iriti M, Hassani L. A Comparative Study of the in Vitro Antimicrobial and Synergistic Effect of Essential Oils from *Laurus nobilis* L. and *Prunus armeniaca* L. from Morocco with Antimicrobial Drugs: New Approach for Health Promoting Products. Antibiotics. 2020 Apr;9(4):140.

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Review Article

The 'black fungus' Co-Infection in COVID-19 Patients : A Review

Jessica Novia¹, Friska Wilda², Alius Cahyadi^{3*}, Marcella Adisuhanto³

¹ Marianum Halilulik Hospital, Belu, East Nusa Tenggara

² Panti Wilasa Citarum Hospital, Semarang

³ Department of Internal Medicine, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia, Jakarta

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ABSTRACT

Mucormycosis is one type of fungal disease, associated with a poor prognosis if not promptly diagnosed and managed because its highly aggressive tendency. Although it is a rare disease, a rapid increase in cases of mucormycosis associated with COVID-19 is being reported. Mostly, risk factors for this disease are uncontrolled diabetes mellitus, other immunosuppressive conditions and corticosteroid therapy. Immune dysfunction, lung pathology and corticosteroid therapy in COVID-19 patients making it more susceptible to develop fungal infection including mucormycosis. The combination of steroid therapy and underlying diabetes mellitus in COVID-19 also can augment immunosuppression and hyperglycemia. Control of hyperglycemia, early treatment with liposomal amphotericin B, and surgery are three important factors in mucormycosis therapy that essential for successful management. However, in this COVID-19 pandemic situation, that management strategies are compromised. First, hyperglycemia can be aggravated by glucocorticoid, therapy that used widely for COVID-19 especially in severe case. Second, patients with ARDS and multiorgan dysfunction can prevent timely diagnostic for imaging and other testing, so appropriate therapy that should be given will be delayed. Last, the essential service in hospital such surgery in this pandemic era reduced significantly to prevent the spread of COVID-19. This review was created with the aim mucormycosis co-infection can be considered in patients with COVID-19, especially with known risk factor. Prompt and rapid diagnosis are important for effective therapy and decreasing case fatality rate. The use of steroid in mild cases, utilization of higher doses of steroid and drugs that targeting immune pathway should be avoided.

Keywords: Mucormycosis; Black Fungus; Coronavirus; COVID-19

ABSTRAK

Mucormycosis merupakan salah satu penyakit infeksi jamur dengan tingkat penularan yang tinggi. Jika tidak segera didiagnosis dan diterapi, maka berhubungan dengan prognosis yang buruk. Walaupun penyakit ini jarang ditemukan, tetapi data penelitian terbaru melaporkan peningkatan signifikan kejadian mucormycosis pada pasien COVID-19. Umumnya, penyakit diabetes melitus yang tidak terkontrol, kondisi immunosupresif lain dan terapi kortikosteroid merupakan faktor risiko terjadinya mucormycosis. Disfungsi sistem imun, kelainan patologi paru dan terapi kortikosteroid pada pasien COVID-19 membuat pasien lebih berisiko untuk mengalami infeksi sekunder termasuk mucormycosis. Kombinasi terapi steroid dan adanya komorbid diabetes melitus pada COVID-19 juga lebih meningkatkan kondisi immunosupresi dan hiperglikemia. Kontrol hiperglikemia, pengobatan awal dengan liposomal amfoterisin B, dan pembedahan adalah tiga aspek penting dalam terapi mucormycosis yang merupakan faktor penentu keberhasilan penatalaksanaannya. Walaupun demikian, dalam situasi pandemi COVID-19 ini, strategi penatalaksanaan tersebut sulit tercapai. Pertama, kondisi hiperglikemia dapat diperburuk dengan glukokortikoid, yang merupakan terapi yang digunakan secara luas untuk COVID-19 terutama pada kasus berat. Kedua, pasien dengan ARDS dan disfungsi multiorgan dapat membuat uji diagnosis seperti pencitraan dan tes lainnya menjadi terlambat dilakukan sehingga diagnosis dan terapi pasien akan tertunda. Terakhir, di era pandemi

* Corresponding Author:
alius.cahyadi@atmajaya.ac.id

ini pelayanan di rumah sakit yang memerlukan tindakan, termasuk operasi berkurang secara signifikan untuk mencegah penyebaran COVID-19 lebih luas. Tujuan penulisan artikel ini adalah agar koinfeksi mucormycosis dapat dipertimbangkan pada pasien dengan COVID-19, terutama dengan faktor risiko yang berkaitan. Diagnosis yang cepat dan tepat penting untuk terapi yang efektif dan dapat menurunkan angka kematian. Penggunaan steroid pada kasus ringan, penggunaan steroid dosis tinggi dan obat-obatan yang menargetkan jalur imunologi harus dihindari.

Kata kunci: Mucormycosis; Jamur Hitam; Coronavirus; COVID-19

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INTRODUCTION

Bacterial and fungal secondary infections are particularly vulnerable to occur in patients with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).¹ While several treatment options have been evaluated, none except systemic glucocorticoids have been shown to improve survival in coronavirus disease of 2019 (COVID-19). Steroids therapy like a double-edged sword that is recommended and frequently given for the treatment of COVID-19 but making patients more vulnerable to secondary bacterial and invasive fungal infections.^{2,3} Mucormycosis, the “Black Fungus”, is increasing among COVID-19 patients, as uncontrolled diabetes mellitus (DM) and the use of steroids during COVID-19 treatment are risk factors for mucormycosis.^{4,5} Mucormycosis is a rare disease, but the unique pandemic conditions make it easier for fungi to infect COVID-19 patients which can lead to high morbidity and mortality. Mucormycosis mortality rate is around 54%, but rates varies for each individual depends on underlying conditions, body site affected, and type of fungus.⁶ The diagnosis of mucormycosis could be done by histopathological or culture examinations; which takes a long time about 10 days after symptoms/signs presentation while the disease tends to spread rapidly throughout the body.⁷ Globally, COVID-19 associated mucormycosis highest cases that estimated more than 4000 people infected has been reported in India.⁸ Recently, due to increasing number of COVID-19 cases, we have seen a rapid increase in cases of mucormycosis that attacks a person's sinuses, lungs, and brain.

In this review, we would like to summarize recent data concerning mucormycosis co-infection in COVID-19 patients, epidemiology, pathogenesis and treatments. Mucormycosis disease progression is rapid and have angioinvasive nature, so a prompt diagnosis and treatment should be started as soon as possible to reduce the mortality.

MUCORMYCOSIS

Mucormycosis, formerly known as zygomycosis is a fungal disease caused by a group of molds called mucormycetes. These diseases are most often caused by a fungus that is found in soil and decaying vegetation, usually inhaled by humans from the air. There are various ways a person can contract mucormycosis such as by spores inhalation, food containing spores consumption, and spores-contaminated wound.^{9–11} This infection is mostly attacking immunocompromised individuals or taking medicines that weakened their immune system. The most common fungal species that result in mucormycosis are the *Rhizopus* species and *Mucor* species.⁶

DISCUSSION

Epidemiology

Globally, mucormycosis prevalence around 10,000 cases in the world except India and after merging with India to become 910,000 cases globally. Mucormycosis found in tropical and subtropical climates, such as Indonesia. Indonesia is a tropical country, warm and

humid, with numerous environmental fungi. Unfortunately, the prevalence in some developing countries, including Indonesia, are still unclear because the cases remain undiagnosed due to difficulty in collecting tissue samples and limited facilities of mycology laboratories.¹² The etiologic agents mostly are *Rhizopus* spp., *Mucor* spp., and *Lichtheimia* (formerly *Ab-sidia* and *Mycocladius*) spp. Genera of other mucorales such as *Rhizomucor*, *Saksenaia*, *Cunninghamella*, and *Apophysomyces* are less common.¹³

Clinical Manifestation of Mucormycosis

Mucormycosis have six major clinical form based on clinical manifestation and anatomic position of the invasion including rhinocerebral, pulmonary, cutaneous, gastrointestinal, disseminated and unusual form such as endocarditis, osteomyelitis, peritonitis and renal infection.¹⁴ The initial symptoms of mucormycosis are non-specific.

The most common form is rhinocerebral mucormycosis. Presentation usually begins with pain and numbness in the eyes and face, followed by conjunctival suffusion and blurred vision. Fever does not occur in almost 50% of cases. Mostly, leukocytosis may occur. If it is not properly dealt, it could spread to the ethmoid sinus into the orbit caused damage to the function of extraocular muscle and proptosis with chemosis. In initial phase of the infected area appears normal and the concomitant progression of the disease becomes erythema with or without edema, then appears purplish and lastly formed eschar blackish necrotic tissue (Figure 1). Infection may also extend to the mouth and cause the formation of a necrotic ulcer on the palate. This finding indicates that the disease has spread.¹⁵

Pulmonary mucormycosis patients usually present with high-grade fever ($>38^{\circ}\text{C}$) and non-productive cough. Less common symptoms such as pleuritic chest pain and dyspnea. In rare circumstances, can present in endobronchial tree and causing airway obstruction. Cutaneous mucormycosis can classified as localized if affect skin and subcutaneous tissue, or deep extension if invades deeper to muscle, tendon or bone.

Typical presentation is necrotic eschar with erythema and induration in surrounding skin. Gastrointestinal mucormycosis is less common type and hard to diagnose in living patients. Most affected organ is stomach followed by colon and ileum. The presentation usually nonspecific such as neutropenic fever and hematochezia. If severe, this disease can invade blood vessels in bowel and resulting in perforation, peritonitis, hemorrhage and sepsis. Disseminated mucormycosis occur when spreading hematogenously to other organs. Commonly, site of spread is brain, but also can found in liver, spleen, heart and other organ. The presentation may vary according to location and degree of tissue invasion in that affected organ.¹⁴



Fig. 1. Clinical presentations of rhinocerebral mucormycosis. (a) Extraoral examination reveals swelling in the left side of the face just below the eye; (b) Intraoral examination reveals necrotic bone with pus discharge in relation to left maxilla (white arrow).¹⁶

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Diagnosis

The diagnostic pathway was designed by the European Confederation of Medical Mycology and the Mycoses Study Group Education and Research Consortium (ECMM/MSGERC) consensus. The ability in diagnosing mucormycosis basically depends on the well-trained staffs, techniques and imaging types, and mycological and histological investigations. A prompt referral to the highest care level was recommended for patients with suspected mucormycosis.¹³

Diagnostic for mucormycosis such as radiologic features is nonspecific and have wide range of types. The most common features that can be identified for pulmonary mucormycosis are presence of nodules, consolidations, reverse halo sign, large perilesional halo (Figure 2) and cavitation. Reverse halo sign characterized by peripheral consolidation with central ground glass and large perilesional halo characterized by ground-glass halo around lesion that very extensive and bigger than the lesion itself.^{17,18}

In rhinocerebral mucormycosis, sinus involvement usually occurs and must be identified in radiologic findings. The most common

paranasal sinus involved are maxillary, ethmoid and sphenoid. Mucosal thickening and bone erosion in imaging also the common features. Signal characteristics and contrast enhancement can be seen in CT scan, Most common form is mild enhancement. Less common, non-enhancing and heterogenous pattern can also be found. If the imaging present with non-enhancing opacification of sinuses, presence of retro antral, facial and orbital fat stranding and hypodense soft tissue extension indicated aggressive infection. (Figure 3). Lastly, imaging must identify extra sinus extension such as orbit and face.¹⁹

Histopathological examination for mucormycosis is important but not always reliable to differentiate with *Aspergillus*. Mucorales have primitive coenocytic hyphae which are fragile because of lack of regular hyphae-septations. They make aggressive tissue grinding can render fragile fungal elements become non-viable. The important differentiation between Mucorales and *Aspergillus* is on their hypha type. Mucorales hypha have wide diameter and non-septate while *Aspergillus* hypha is narrower and have many septation.^{20,21}

Imaging

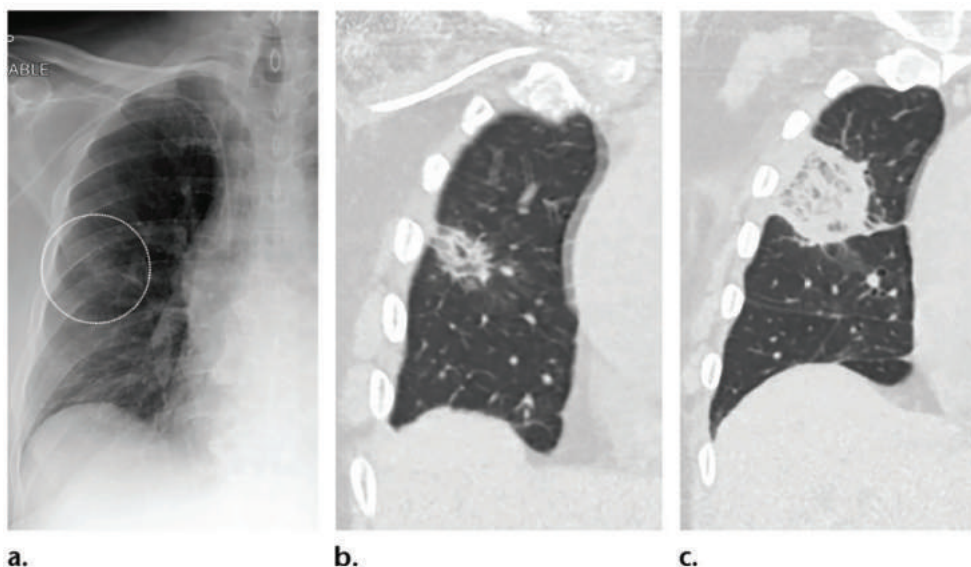


Fig. 2 (a) Frontal radiograph of the right lung shows a faint area of ground-glass opacity (dotted circle); (b) Coronal CT image obtained an area of nodular ground-glass opacity; (c) Coronal CT image shows enlargement of the lesion with development of the reverse halo sign.²²

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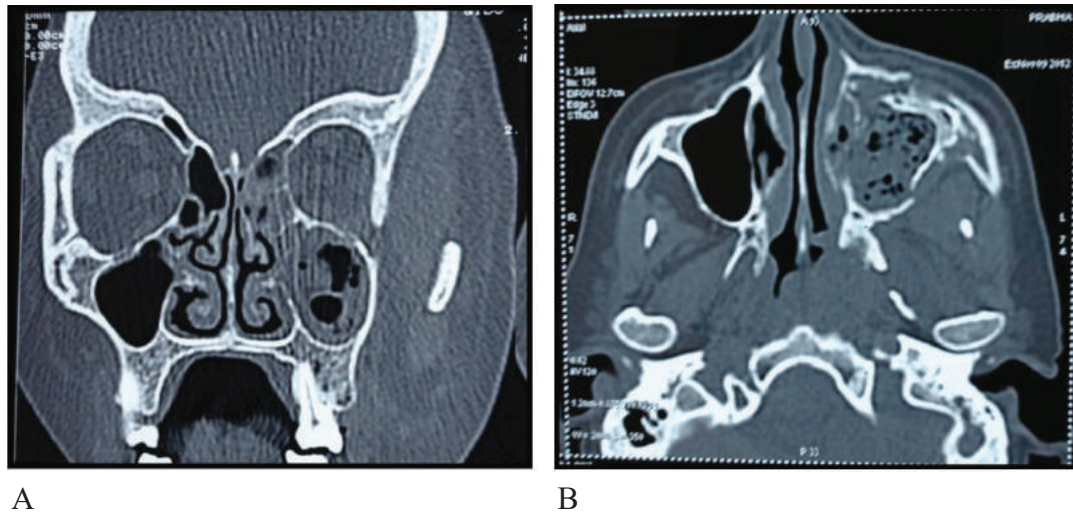


Fig. 3 (a) Coronal view of CT showing involvement of left maxillary sinus, nasal conchae, and ethmoidal sinus extending up to frontal sinus; (b) Axial view of CT showing destruction of posterior, medial, and anterior walls of left maxillary sinus.¹⁶
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Histopathology in Mucormycosis

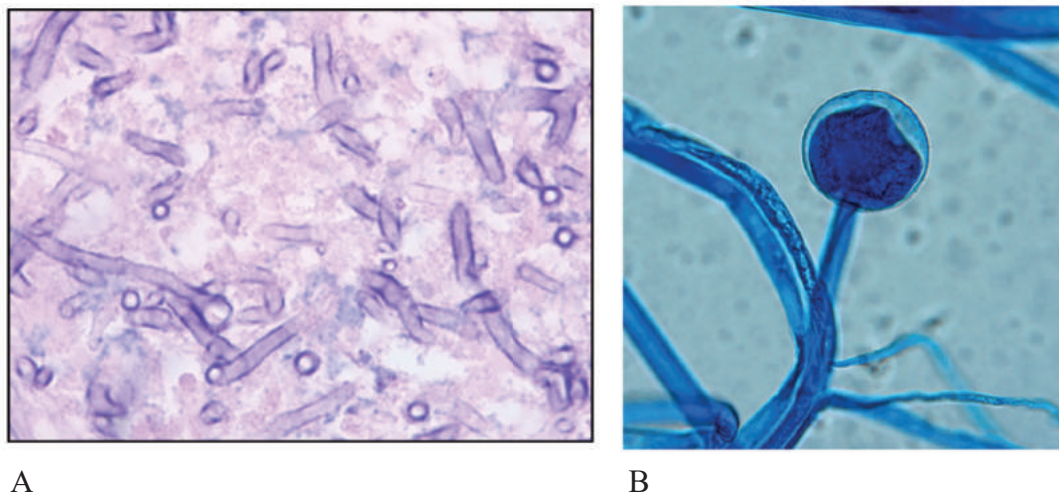


Fig. 4. Structure of *Mucor*. (a) Mucorales are irregular hyphae with wide width (6 to 25-micron diameter) are non-septate or sparsely septate, ribbon-like; (b) High-power photomicrograph shows a spherical structure called the sporangium. (Lactophenol cotton blue stain).^{22,23}
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Culture and Microscopy

Culture is highly recommended for identification of fungal genus and species.⁷ It should be noted that culture does not always work for several reasons, including improper sampling and incorrect sample treatment before the examination. In fact, only 15-25% of cases are positive.²⁴

Treatment

A multimodal approach is needed in the management of mucormycosis, such as discontinuation of risk factors, early administration of antifungal therapy with optimal doses, and surgical intervention. Treatment should be started immediately if the diagnosis is suspected because the disease tends to spread rapidly throughout the

body, although the exact diagnosis has not been confirmed.¹³

Prophylaxis

Posaconazole delayed-release tablets are recommended for neutropenic patients or those with graft versus host disease.⁷

First-line antifungal monotherapy

Daily doses of liposomal amphotericin B ranged 5-10 mg/kg for any patients and ≤ 5 mg/kg if renal toxicity develops. In Central Nervous System (CNS) involvement, use of amphotericin B lipid complex 5 mg/kg per day. Treatment duration given usually weeks to months, depending on each patients condition . If the immune defect is resolved, such as well-controlled diabetes and resolved neutropenia, immunosuppressant can be tapered or stopped, therapy can be continued until resolution of signs and symptoms of infection, and substantial radiographical improvement.⁷ At the fourth week, the overall response rate was 36%, while in the twelfth week, the overall response was 45%.¹³

First-line antifungal combination monotherapy

There are no definitive data to guide the use of antifungal combination therapy.⁷

Antifungal salvage treatment

Daily Isavuconazole 200mg (after six doses of 200 mg q8h) and Posaconazole delayed-release tablets at a dose of 200 mg q6h or infusions are strongly supported as salvage treatment.^{7,13}

Surgery

Aggressive surgery is often required not only on necrotic tissue but also on surrounding tissue that appears healthy because the Mucorales grow so rapidly.⁷

THE LINK BETWEEN MUCORMYCOSIS CO-INFECTION COVID-19

As previously stated that mucormycosis is mainly attacking immunocompromised patients,

although possibly found in immunocompetent individuals.^{25,26} Generally, mucormycosis does not pose a serious threat to healthy individuals because immune system mainly polymorphonuclear cells can destroy the spores and hyphae.²⁶⁻²⁸ When patients are exposed to SARS-CoV-2, the virus will target the immune system. The relationship between COVID-19 and mucormycosis is the state of weakened of patients' immune responses, with reduced numbers of T lymphocytes, CD4+, and CD8+ T cells and medical treatment with a steroid to reduce inflammation.²⁹

One of the major risk factors that increasing morbidities and mortalities in COVID-19 associated with mucormycosis, is diabetes mellitus.³⁰⁻³² In patients with diabetes, *Rhizopus* is the most commonly found fungus. The reason which allows them to survive in high acid and glucose are an enzyme properties and ketone reductase.³³ Treatment pathway for patient with COVID-19 with mucormycosis co-infection including both diseases therapy. Therapy requires surgical debridement, antifungal treatment and stabilization of risk factor.⁸

Diabetic ketoacidosis (DKA) often occurs in severe infections, such as in COVID-19. Therefore, it is not surprising that patients with COVID-19 are more likely to develop mucormycosis because acidic conditions make mucorales species easier to grow.³⁴ Research suggests SARS-CoV-2 induces damage of pancreatic islets resulting in acute diabetes and DKA.³⁵ Another explanation for why the diabetogenic state occurs in patients with severe COVID-19 is due to cytokine storms that increase insulin resistance and high expression of angiotensin-converting enzyme 2 receptors in pancreatic islets. Increased serum ferritin levels in severe COVID-19 also one of the possible roles of blood acidosis for mucormycosis susceptibility.^{34,36-38}

It has been proven that by administration of systemic corticosteroids could cut down death rates in COVID-19 patients on invasive mechanical ventilation.^{39,40} According to the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium

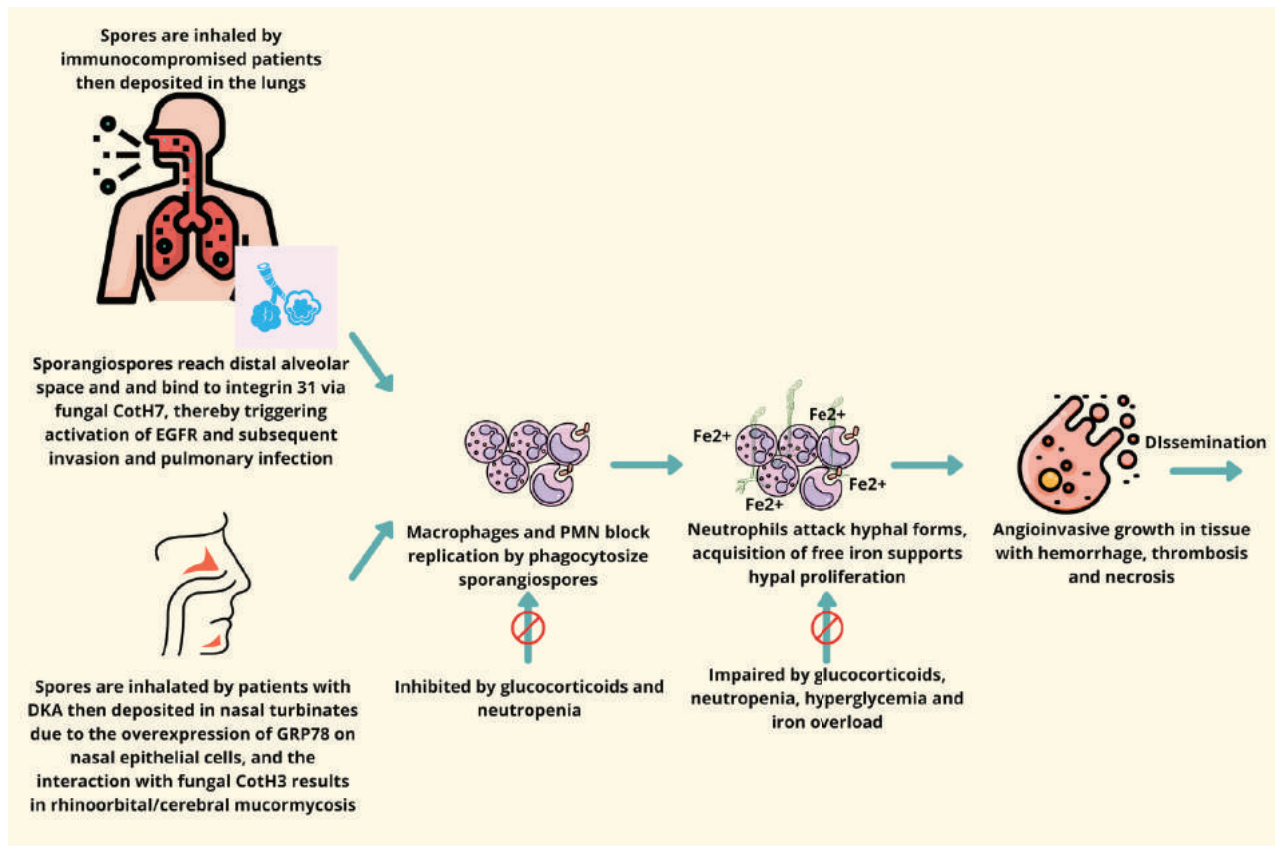


Fig. 5 Pathogenesis of Mucormycosis^{44,45}

(EORTC/MSGERC) consensus, long term corticosteroids at a therapeutic dose of ≥ 0.3 mg/kg for at most three weeks in the past 60 days is considered a risk factor for invasive fungal diseases.^{41,42} The use of corticosteroids can also increase blood glucose levels to hyperglycemia even though the individual is healthy, causing the condition called corticosteroid-induced diabetes. This corticosteroid and the diabetogenic state later can cause immunosuppression and hyperglycemia, increasing the growth of fungal infections, including mucormycosis (figure 5).^{3,5,43}

Iron acquisition is a critical step that occurs in severe COVID-19. Members of the class Zygomycetes are the only fungus identified that stores iron in ferritins. The problem is tissue damage can occur in high ferritin levels. High ferritin levels lead to excess intracellular iron that generates reactive oxygen species. Ferritin synthesis and downregulate iron export can also occur due to severe infection and DKA.^{46–50}

The resultant tissue damage leads to the release of free iron into the circulation, which further exacerbates the mucormycosis process.⁵¹

“Endothelialitis” in patients with severe COVID-19 is also one of the associations between COVID-19 and mucormycosis.^{52,53} Important initial steps of mucormycosis are endothelial adhesion and penetration.⁷ In addition, acidemic states, and hyperglycemia induce the endothelial receptor glucose-regulated protein (GRP 78) and the mucorales adhesin spore coat protein homologs (CotH), creating a “perfect storm” for increased adhesion and penetration of mucorales to the endothelium.⁵⁴

Based on the available literature regarding mucormycosis co-infection COVID-19, there were six studies reporting 28 patients that have reported rhino-orbito-cerebral mucormycosis. It is important to remember that mucormycosis can occur at any time after a COVID-19 infection, either during the hospital stay, or a few days to weeks after discharge. Therefore, all physicians

could be more aware of these side effects of the kinds of treatment patients are given and how could patients be more aware of what they could face because of the medicine that they are taking, especially if having underlying conditions. They should knowledgeable about the red flag symptoms of invasive mucormycosis.^{11,29,55–58}

Alekseyev, et al presented a 41-year-old man with a history of type 1 diabetes mellitus (T1DM), COVID-19 pneumonia and rhinocerebral mucormycosis. He was treated with steroids and hydroxychloroquine before, as the recommended regional COVID-19 practice guideline at the time. For his diabetic ketoacidosis (DKA) treated with intravenous fluids and an intravenous insulin, cefepime and amphotericin B IV, along with three surgical debridements for the rhinocerebral mucormycosis. The patient successfully discharged and continued the treatment at home.⁵⁹

Another study by *Kanwar et al*, they presented 56-year-old man with COVID-19 and underlying end-stage renal disease. This patients also developed mucormycosis as a complication of COVID-19. He received a five-day therapy of methylprednisone, one dose of tocilizumab, and one unit of convalescent plasma. At first hospital admission, blood cultures collected were negative for bacterial and fungal organisms. He was discharged home seven days later but five days later he was readmitted because shortness of breath. Polymerase chain reaction (PCR) examination for COVID-19 was positive again and chest radiograph showed increasing density and pleural effusion. He was started on empiric intravenous (IV) vancomycin and piperacillin-tazobactam. Sputum sample was collected and showed filamentous fungal elements on fungal stain that was suspected from Mucorales group because non-septate hyphae. Empiric amphotericin B was started and antibacterial medications were discontinued, unfortunately the patient developed cardiac arrest and died the following day.⁶⁰

Maini et al, reported a 38-year-old man with COVID-19 confirmed, no history of diabetes or

other condition. He was monitored in ICU and started on remdesivir IV, methylprednisolone IV and dexamethasone. After 12 days of treatment, the glycated hemoglobin (HbA1C) level was 12.3%. Eighteen days later, the patient complaint of swelling and pain in his left eye, then underwent MRI scan and histopathologic examination from sinus sample. Patient was then diagnosed as sino-orbital mucormycosis. Medical treatment was changed into amphotericin B and patient was going into surgical debridement. After a total of 38 days of hospitalization, he was discharged and continued treatment at home.⁶¹

CONCLUSION

In COVID-19, due to immune system dysregulation, diabetogenic state, endothelialitis, and the widespread use of steroids as therapy against COVID-19 may lead to the development/exacerbation of pre-existing fungal diseases. Physicians should be aware of the development/exacerbation of pre-existing fungal infection among COVID-19 patients, especially if rhino-orbital-cerebral presentations are noted. A multidisciplinary approach should include the recognition of host factors, assessment of clinical manifestations, use of appropriate imaging modalities, histology and microbiology with any appropriate surgical consultation and treatment. The use of steroids should be monitored to achieve a therapeutic effect at the lowest dose and shortest durations to lower the risk of development/exacerbation of pre-existing fungal infection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Clancy CJ, Schwartz IS, Kula B, Nguyen MH. Bacterial Superinfections Among Persons With Coronavirus Disease 2019: A Comprehensive Review of Data From Postmortem Studies. *Open Forum Infect Dis*. 2021;8(3).
- Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, et al. COVID-19 associated pulmonary aspergillosis (CAPA)—from immunology to treatment. *J Fungi*. 2020;6(2):1–17.
- Ardi P, Daie-Ghazvini R, Hashemi SJ, Salehi MR, Bakhshi H, Rafat Z, et al. Study on invasive aspergillosis using galactomannan enzyme immunoassay and determining antifungal drug susceptibility among hospitalized patients with hematologic malignancies or candidates for organ transplantation. *Microb Pathog*. 2020 Oct;147:104382.
- Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, et al. Coronavirus Disease (Covid-19) Associated Mucormycosis (CAM): Case Report and Systematic Review of Literature. *Mycopathologia*. 2021;2.
- Lionakis MS, Kontoyiannis DP. Glucocorticoids and invasive fungal infections. Vol. 362, *Lancet*. Elsevier B.V.; 2003. p. 1828–38.
- Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: A review of 929 reported cases. *Clin Infect Dis*. 2005;41(5):634–53.
- Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. *Lancet Infect Dis*. 2019;19(12):e405–21.
- WHO. Epidemiological Alert: COVID-19 associated Mucormycosis . *Pan Am Heal Organ* . 2021; Available from: https://iris.paho.org/bitstream/handle/10665.2/54284/EpiUpdate11June2021_eng.pdf?sequence=1&isAllowed=y
- Mucormycosis | Fungal Diseases | CDC.
- Richardson M. The ecology of the zygomycetes and its impact on environmental exposure. *Clin*
- Werthman-Ehrenreich A. Mucormycosis with orbital compartment syndrome in a patient with COVID-19. *Am J Emerg Med*. 2021 Apr;42:264.e5-264.e8.
- Prakash H, Chakrabarti A. Global epidemiology of mucormycosis. *J Fungi*. 2019;5(1).
- Skiada A, Lass-Floerl C, Klimko N, Ibrahim A, Roilides E, Petrikos G. Challenges in the diagnosis and treatment of mucormycosis. *Med Mycol*. 2018;56:S93–101.
- Petrikos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. *Clin Infect Dis [Internet]*. 2012 Feb 1 [cited 2021 May 30];54(SUPPL. 1):S23–34. Available from: https://academic.oup.com/cid/article/54/suppl_1/S23/284492
- ASI. BS. Mucormycosis | Harrison's Principles of Internal Medicine, 19e | AccessMedicine | McGraw-Hill Medical. 2015. 1350–53 p.
- Garlapati K, Chavva S, Vaddeswarupu RM, Surampudi J. Case Report Fulminant Mucormycosis Involving Paranasal Sinuses: A Rare Case Report. 2014;
- Peng M, Meng H, Sun Y, Xiao Y, Zhang H, Lv K, et al. Clinical features of pulmonary mucormycosis in patients with different immune status. *J Thorac Dis [Internet]*. 2019 Dec 1 [cited 2021 May 31];11(12):5042–52. Available from: [/pmc/articles/PMC6988080/](https://pubmed.ncbi.nlm.nih.gov/34111111/)
- Hammer MM, Madan R, Hatabu H. Pulmonary mucormycosis: Radiologic features at presentation and over time. *Am J Roentgenol*. 2018 Apr;210(4):742–7.
- Therakathu J, Prabhu S, Irodi A, Sudhakar SV, Yadav VK, Rupa V. Imaging features of rhinocerebral mucormycosis: A study of 43 patients. *Egypt J Radiol Nucl Med*. 2018 Jun;49(2):447–52.
- Skiada A, Lass-Floerl C, Klimko N, Ibrahim A, Roilides E, Petrikos G. Challenges in the diagnosis and treatment of mucormycosis [Internet]. Vol. 56, *Medical Mycology*. Oxford University Press; 2018 [cited 2021 May 31]. p. S93–101. Available from: https://academic.oup.com/mmy/article/56/suppl_1/S93/4925966
- Kimura M, Nishimura K, Enoki E, Chikugo T, Maenishi O. Chlamyospores of *Rhizopus microsporus* var. *rhizopodiformis* in Tissue of Pulmonary Mucormycosis.
- R A, A Y, H S, ND P, PJ L, EM H. Pulmonary Mucormycosis: Risk Factors, Radiologic Findings, and Pathologic Correlation. *Radiographics*. 2020 May;40(3):656–66.
- Mekki SO, Hassan AA, Falemban A, Alkotani N, Alsharif SM, Haron A, et al. Pulmonary Mucormycosis: A Case Report of a Rare Infection with Potential Diagnostic Problems. *Case Rep Pathol*. 2020 Jan;2020:1–4.
- Lass-Flörl C. Zygomycosis: Conventional laboratory diagnosis. *Clin Microbiol Infect*. 2009;15(SUPPL. 5):60–5.
- Spellberg B, Ibrahim AS, Chin-Hong PV, Kontoyiannis DP, Morris MI, Perfect JR, et al. The deferasirox-AmBisome therapy for mucormycosis (Defeat Mucor) study: A randomized, double-blinded, placebo-controlled trial. *J Antimicrob Chemother*. 2012;67(3):715–22.
- Riley TT, Muzny CA, Swiatlo E, Legendre DP. Breaking the Mold: A Review of Mucormycosis and Current Pharmacological Treatment Options. Vol. 50, *Annals of Pharmacotherapy*. SAGE Publications Inc.; 2016. p. 747–57.

27. Ferguson BJ. Mucormycosis of the nose and paranasal sinuses. *Otolaryngol Clin North Am.* 2000 Apr;33(2):349–65.
28. Greenberg RN, Scott LJ, Vaughn HH, Ribes JA. Zygomycosis (mucormycosis): Emerging clinical importance and new treatments. Vol. 17, *Current Opinion in Infectious Diseases.* Curr Opin Infect Dis; 2004. p. 517–25.
29. Mehta S, Pandey A. Rhino-Orbital Mucormycosis Associated With COVID-19. *Cureus.* 2020;12(9):10–4.
30. Garg D, Muthu V, Sehgal IS, Ramachandran R, Kaur H, Bhalla A, et al. Coronavirus Disease (Covid-19) Associated Mucormycosis (CAM): Case Report and Systematic Review of Literature. *Mycopathologia.* 2021;186(2):289–98.
31. Lansbury LE, Rodrigo C, Leonardi-Bee J, Nguyen-Van-Tam J, Lim WS. Corticosteroids as adjunctive therapy in the treatment of influenza: An updated cochrane systematic review and meta-analysis. *Crit Care Med.* 2020;E98–106.
32. Afroze SN, Korlepara R, Rao GV, Madala J. Mucormycosis in a diabetic patient: A case report with an insight into its pathophysiology. *Contemp Clin Dent.* 2017 Oct;8(4):662–6.
33. Nagao K, Ota T, Tanikawa A, Takae Y, Mori T, Udagawa SI, et al. Genetic identification and detection of human pathogenic *Rhizopus* species, a major mucormycosis agent, by multiplex PCR based on internal transcribed spacer region of rRNA gene. *J Dermatol Sci.* 2005 Jul;39(1):23–31.
34. Spellberg B, Edwards J, Ibrahim A. Novel perspectives on mucormycosis: Pathophysiology, presentation, and management. Vol. 18, *Clinical Microbiology Reviews.* 2005. p. 556–69.
35. Yang JK, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. *Acta Diabetol.* 2010;47(3):193–9.
36. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct Target Ther.* 2020;5(1):16–8.
37. Rammaert B, Lanternier F, Poirée S, Kania R, Lortholary O. Diabetes and mucormycosis: A complex interplay. Vol. 38, *Diabetes and Metabolism.* Elsevier Masson; 2012. 38(3):193–204.
38. Balasopoulou A, Kokkinos P, Pagoulatos D, Plotas P, Makri OE, Georgakopoulos CD, et al. Symposium Recent advances and challenges in the management of retinoblastoma Globe - saving Treatments. *BMC Ophthalmol.* 2017;17(1):1.
39. Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, Angus DC, et al. Association between Administration of Systemic Corticosteroids and Mortality among Critically Ill Patients with COVID-19: A Meta-analysis. *JAMA - J Am Med Assoc.* 2020;324(13):1330–41.
40. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med.* 2021;384(8):693–704. Peter Donnelly J, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, *et al.* Revision and update of the consensus definitions of invasive fungal disease from the european organization for research and treatment of cancer and the mycoses study group education and research consortium. *Clin Infect Dis.* 2020;71(6):1367–76.
41. Song Y, Zhang M, Yin L, Wang K, Zhou Y, Zhou M, et al. COVID-19 treatment: close to a cure? A rapid review of pharmacotherapies for the novel coronavirus (SARS-CoV-2). Vol. 56, *International Journal of Antimicrobial Agents.* Elsevier B.V.; 2020.
42. Aljehani M, Alahmadi H, Alshamani M. Case Report A Case Report of Complete Resolution of Auricular Mucormycosis in an 18-Month-Old Diabetic Child. 2021;
43. Alqarihi A, Gebremariam T, Gu Y, Swidergall M, Alkhazraji S, Soliman SSM, et al. GRP78 and integrins play different roles in host cell invasion during mucormycosis. *MBio.* 2020;11(3).
44. Lewis RE, Kontoyiannis DP. Epidemiology and treatment of mucormycosis. *Future Microbiology.* 2013;8(9):1163–75.
45. Perricone C, Bartoloni E, Bursi R, Cafaro G, Guidelli GM, Shoenfeld Y, et al. COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy. *Immunol Res.* 2020;68(4):213–24.
46. De Locht M, Boelaert JR, Schneider YJ. Iron uptake from ferrioxamine and from ferrirhizoferrin by germinating spores of *rhizopus microsporus*. *Biochem Pharmacol.* 1994;47(10):1843–50.
47. Maertens J, Demuynck H, Verbeke EK, Zachée P, Verhoef GEG, Vandenberghe P, et al. Mucormycosis in allogeneic bone marrow transplant recipients: Report of five cases and review of the role of iron overload in the pathogenesis. *Bone Marrow Transplant.* 1999;24(3):307–12.
48. Cardoso F, Senkus E, Costa A, Papadopoulos E, Aapro M, André F, et al. 4th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 4). *Ann Oncol.* 2018;29(8):1634–57.
49. Boelaert JR, De Locht M, Van Cutsem J, Kerrels V, Cantinieaux B, Verdonck A, et al. Mucormycosis during deferoxamine therapy is a siderophore-mediated infection: In vitro and in vivo animal studies. *J Clin Invest.* 1993;91(5):1979–86.
50. Edeas M, Saleh J, Peyssonnaud C. Iron: Innocent bystander or vicious culprit in COVID-19 pathogenesis? *Int J Infect Dis.* 2020;97:303–5.
51. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med.* 2020 Jul;383(2):120–8.
52. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell

- infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417–8.
53. Sabirli R, Koseler A, Goren T, Turkcuer I, Kurt O. High GRP78 levels in Covid-19 infection: A case-control study. *Life Sci*. 2021;265(October 2020):118781.
54. Moorthy A, Gaikwad R, Krishna S, Hegde R, Tripathi KK, Kale PG, et al. SARS-CoV-2, Uncontrolled Diabetes and Corticosteroids—An Unholy Trinity in Invasive Fungal Infections of the Maxillofacial Region? A Retrospective, Multi-centric Analysis. *J Maxillofac Oral Surg*. 2021;2.
55. Sarkar S, Gokhale T, Choudhury S, Deb A. COVID-19 and orbital mucormycosis. Vol. 69, *Indian Journal of Ophthalmology*. Wolters Kluwer Medknow Publications; 2021. p. 1002–4.
56. Waizel-Haiat S, Guerrero-Paz JA, Sanchez-Hurtado L, Calleja-Alarcon S, Romero-Gutierrez L. A Case of Fatal Rhino-Orbital Mucormycosis Associated With New Onset Diabetic Ketoacidosis and COVID-19. *Cureus*. 2021 Feb;13(2).
57. Mekonnen ZK, Ashraf DC, Jankowski T, Grob SR, Vagefi MR, Kersten RC, et al. Acute Invasive Rhino-Orbital Mucormycosis in a Patient with COVID-19-Associated Acute Respiratory Distress Syndrome. *Ophthal Plast Reconstr Surg*. 2021;37(2):E40–2.
58. Alekseyev K, Didenko L, Chaudhry B. Rhinocerebral Mucormycosis and COVID-19 Pneumonia. *J Med Cases [Internet]*. 2021 [cited 2021 May 31];12(3):85–9. Available from: [/pmc/articles/PMC8040444/](https://pubmed.ncbi.nlm.nih.gov/34844444/)
59. Kanwar A, Jordan A, Olewiler S, Wehberg K, Cortes M, Jackson BR. A Fatal Case of *Rhizopus azygosporus* Pneumonia Following COVID-19. *J Fungi [Internet]*. 2021 [cited 2021 May 31];7:174. Available from: <https://doi.org/10.3390/jof7030174>
60. Maini A, Tomar G, Khanna D, Kini Y, Mehta H, Bhagyasree V. Sino-orbital mucormycosis in a COVID-19 patient: A case report. *Int J Surg Case Rep*. 2021 May 1;82:105957.



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Chapter in a book

4. Vidyadaran S, Ramasamy R, Seow HF. Stem cells and cancer stem cells: Therapeutic Applications in Disease and Injury. In: Hayat MA, editor. New York: Springer; 2012.

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5. Canadian Dental Hygienists Association. Dental hygiene: definition and scope. Ottawa: Canadian Dental Hygienists Association; 1995.

E-book

6. Frank SA. Immunology and Evolution of Infectious Disease [Internet]. Princeton: Princeton University Press; 2002 [cited 2014 December 17]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK2394/pdf/TOC.pdf>

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2. Abdullah M, Chai PS, Chong MY, Tohit ERM, Ramasamy R, Pei CP, et al. Gender effect on in vitro lymphocyte subset levels of healthy individuals. Cellular Immunology. 2012;272(2):214-9.

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3. Clancy JL, Patel HR, Hussein SM, Tonge PD, Cloonan N, Corso AJ, et al. Small RNA changes enroute to distinct cellular states of induced pluripotency. Nature communications.2014; 5:5522. Epub 2014/12/11.

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