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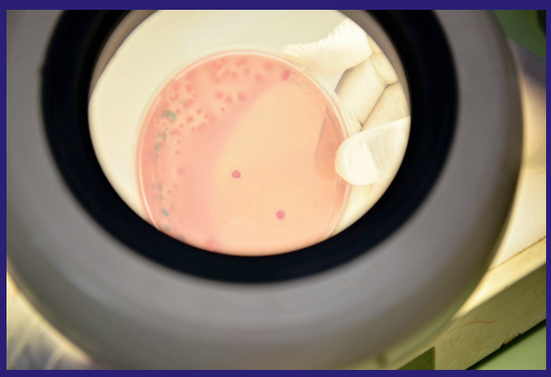




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# *Indonesian Journal of* Tropical and Infectious Disease

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**Review Article**

# Novel Potential Immune Response Biomarkers to Multidrug-Resistant Tuberculosis in the Last Five Years

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***ABSTRACT***

*Rapid and accurate detection performs an important role in the control of raising MDR-TB. Currently, studies on biomarkers as targets for TB diagnostic tests using immune response products to indicate the presence, mycobacterial load, early markers, and activity, differentiation, and progression markers of TB infection are rapidly available. This systematic review aims to summarize the last five years of potential biomarkers studies from the immune response for MDR-TB rapid diagnostic development. The authors performed a literature search on four databases as ProQuest, EBSCO Academic Search, Universitas Gadjah Mada Online Library Journal Database, and Google Scholar, retrieved from January 2016 to December 2021. In total, 18,288 articles were identified and three tudies met the inclusion criteria. Several promising biomarkers were found for MDR-TB diagnosis purposes, such as sCD14, PGLYRP2, FGA, Indoleamine 2, 3- dioxygenase (IDO), and Complement Receptor 2 (CR2). A combination of sCD14, PGLYRP2, and FGA were bringing a diagnostic design with a higher sensitivity (94.7%) and specificity (80%) than the design of a single protein. Higher IDO activity towards the MDR-TB group than in the DS-TB group with a sensitivity of 87.50 %, specificity of 72.22 %. CR2 was the main focus due to its association with IL-6. After induction of CR2 peptide in a dose-dependent manner, the expression level of IL-6 was decreased significantly. It might because of CR2 peptide regulating the macrophages proinflammatory cytokines secretion to decrease the local inflammation of the immune response. These biomarkers are strong candidates for MDR-TB diagnosis due to their important role as the pathogenesis marker of MDR-TB. There is a need of further research to investigate those immune response products and their role to eliminate infection of Mycobacterium tuberculosis directly.*

***Keywords:*** *Biomarkers, diagnosis, immune response, multidrug resistant, tuberculosis.*

***ABSTRAK***

*Deteksi cepat dan akurat berperan penting dalam pengendalian MDR-TB. Saat ini, studi tentang biomarker sebagai target untuk tes diagnostik TB menggunakan produk respon imun untuk menunjukkan keberadaan, bacterial load, penanda tahap awal infeksi, aktivitas, diferensiasi, dan penanda perkembangan infeksi TB tersedia dengan cepat. Tujuan dari tinjauan sistematis ini adalah merangkum studi biomarker yang berpotensi selama lima tahun terakhir dari respon imun untuk mengembangkan diagnostik cepat MDR-TB. Penulis melakukan pencarian literatur pada empat database seperti ProQuest, EBSCO Academic Search, Universitas Gadjah Mada Online Library Journal Database, dan Google Scholar dari Januari 2016 hingga Desember 2021. Total terdapat 18.288 artikel yang diidentifikasi dan terdapat 3 artikel yang meenuhi kriteria inklusi. Ditemukan beberapa biomarker potensial untuk diagnosis MDR-TB, seperti sCD14, PGLYRP2, FGA, Indoleamine 2,3- dioxygenase (IDO), dan Complement Receptor 2 (CR2). Kombinasi sCD14, PGLYRP2, dan FGA menghasilkan desain diagnostik dengan sensitivitas (94.7%) dan spesifisitas (80%) yang lebih tinggi dibandingkan desain protein tunggal. Aktivitas IDO lebih tinggi pada kelompok MDR-TB dibandingkan kelompok DS- TB dengan sensitivitas 87.50% dan spesifisitas 72.22%. CR2 menjadi fokus utama karena aktivitasnya terkait dengan IL- 6. Setelah induksi peptide CR2 dengan pemberian dosis terikat, tingkat ekspresi IL- 6 menurun secara signifikan. Hal ini mungkin karena peran peptide CR2 dalam mengatur sekresi sitokin proinflamasi*

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*makrofag untuk mengurangi peradangan lokal dari respon imun. Biomarker- biomarker tersebut merupakan kandidat kuat sebagai biomarker dalam mendiagnosa MDR-TB karena perannya yang penting sebagai penanda patogenesis MDR-TB. Diperlukan penelitian lebih lanjut untuk mengetahui produk respon imun tersebut dan peranannya dalam mengeliminasi infeksi Mycobacterium tuberculosis secara langsung.*

*Kata kunci: Biomarka, diagnosis, multidrug resistant, respon imun, tuberkulosis.*

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## INTRODUCTION

Tuberculosis (TB) caused by the bacterial pathogen *Mycobacterium tuberculosis* is a chronic infection that affects the respiratory system, especially the lungs, and invades various tissues and organs, such as bones and central nervous system.[1–3] TB can be passed from an individual to others through droplets when coughing, sneezing, or conversing. Currently, there are 10.4 million new cases of TB over the globe with a total of 1.7 million deaths since 2013 through nowadays.[4,5]

Multidrug-resistant tuberculosis (MDR-TB) becomes a further problem of TB infection. These conditions include failure to respond to TB main therapy isoniazid and rifampicin, second-line therapy, short-term MDR-TB regimens, and long-term MDR-TB regimens.[3,6,7] The World Health Organization (2019) estimated that currently there are 484,000 MDR-TB cases in the world. The presence of *rpoB* gene mutation which arose due to insufficient administration of drug dose or incomplete therapy is strongly proven as the cause of MDR-TB.[8] Meanwhile, elimination of TB and MDR-TB depends on a wider scope of rapid diagnosis and treatments strategies. MDR-TB infection that has not been diagnosed early is harder to treat and has a high potential to infect a wide range of healthy individuals. This could lead to a high prevalence of MDR-TB, decrease the treatment stride, increased prevalence, new cases, and mortality from TB and MDR-TB. Therefore, rapid and accurate detection play an important role in the control of raising MDR-TB.[4,9,10]

However, clinical diagnosis for MDR-TB is progressing slowly. On the other hand, the efficiency of early detection is low.[11,12] Sputum-based diagnostic tests for active TB patients, such as patients with immunocompromise, diabetes, and child-aged, encounter problems of false-negative tests results.[13,14] In addition, sputum examination is less effective in patients with extrapulmonary tuberculosis. Invasive measures, such as taking samples from tissue or biological fluids, are needed to confirm the diagnosis. Interferon-gamma release assay (IGRA) test, for example Quantiferon-TB gold plus (QFT-Plus), is a rapid diagnostic test for active TB and latent TB that has a sensitivity of 94.1% and specificity of 97.3%.[8,14,15] But this test is infirm in diagnosing for drug-resistant TB/DR-TB. On the other hand, the Xpert MTB/RIF assay is a molecular based on RT-PCR test method which is time-saving to diagnose rifampicin resistant pulmonary TB.[11,16] This test has a sensitivity of 89% and a specificity of 99% for examination of sputum in pulmonary TB patients. Nonetheless, this test shows inconsistent accuracy results in extrapulmonary TB examinations.[12,14,16,17] Therefore, new diagnostic test innovations that could accurately predict active TB, latent TB infection (LTBI), and DR-TB are becoming priority needs nowadays.

Currently, studies on biomarkers as targets for TB diagnostic tests are rapidly available, notably the biomarkers from immune response products. The immune response to tuberculosis infection will produce secreted proteins such as cytokines as the marker of this disease progression in individuals.[18] Individual immune response products could play a role as biomarkers that indicate the presence, mycobacterial load, early markers, and activity, differentiation, and progression markers of TB infection. These

products are expressed with variation in the level, which could be used as benchmark and measurement to denote the progressivity of TB infection as active, latent, or drug resistant.[19–23]

Various studies focus on new discovered immune response products that have the potential to be used as biomarkers of MDR-TB. These biomarkers can be used as candidates for more efficient and accurate MDR-TB diagnostic targets.[14,24] This systematic review aims to summarize the last five years of potential biomarkers studies from immune response products for MDR-TB rapid diagnostic development.

## METHODS

ProQuest, EBSCO Academic Search, Universitas Gadjah Mada Online Library Journal Database, and Google Scholar free web search engine were used to search relevant academic/ articles journals. Relevant articles were in English from the last five years January 1st, 2016 through 10th December, 2021. The searching process used "*Biomarkers* or *biological markers* or *biomarker* or *biological marker,*" "*drug resistance or antibiotic resistance*," and "*multidrug resistant tuberculosis* or *multidrug resistance tuberculosis* or *drug resistance tuberculosis*" as keywords. The inclusion and exclusion criteria were defined as below.

This study included the articles in English, original articles, experimental, and or observational studies, human or in vivo subjects, focused on immune response products, such as cytokines, and multidrug- resistant tuberculosis studies. This study excluded the studies that were conducted before 2016, only focused on tuberculosis without mentioned multidrug-resistant tuberculosis information, drug-susceptible tuberculosis, the study of therapy interventions, a geospatial or epidemiology studies, a clinical trials, retrospective, and systematic reviews.

Specific searched by subject thesaurus terms as "*Tuberculosis,*" "*tropical disease,*" "*Mycobacterium tuberculosis* drug effects," "*Mycobacterium tuberculosis,*" "*multidrug resistance,*" "*life science & biomedicine,*" "*infectious disease,*" "*immune response,*" "*drug resistance in microorganisms,*" "*drug resistance,*" "*diagnostic systems*," "*diagnosis,*" "*cytokines,*" "*biotechnology,*" "*biomarkers,*" "*biochemistry & molecular biology,*" "*antitubercular agents,*" "*antimicrobial resistance,*" "*antimicrobial agents,*" and "*antibiotic resistance*" were used. Titles and abstracts of all collected articles were screened with unblinded names of the articles' authors. The first author discussed with two co-authors when meeting any uncertainty during the screening process. The quality of articles was defined by indexed journal by Scopus minimum on quartile-2 (Q2).

## RESULTS AND DISCUSSION

In total, 18,288 articles were identified. Of those, three studies met the inclusion criteria after a thorough screening process as included in this systematic review studies (Figure 1). The three selected studies are summarized in Table 1.

### sCD14, PGLYRP2, and FGA

Soluble CD14 (sCD14) is reckoned as a good biomarker for activation marker of monocyte-macrophage. Increased level of sCd14 in plasma are often e related to poor prognosis of chronic infection.[25] Thus, this biomarker is used as strong predictor for morbidity and mortality.[26,27] Protein sCD14 plays a role in monocyte activation. In the early stage of TB infection, monocyte migrates to the infection locale and evolves as macrophages which may cause immune responses. Meanwhile, sCD14 level on the other infection, as example respiratory or lung disease, was increasing based on observation. Due to mass spectrometry strategy, sCD14 profusion in the MDR-TB group was lesser than in the DS-TB group, even it was upregulated in both. The possible reason of sCD14 decrease was decreasing of monocyte activation.[25,26,28]

**Table 1.** Summary of reviewed studies

| | Title | Setting |
|---|---|---|
| **Chen et al (2020)** | Serum sCD14, PGLYRP2 and FGA as potential biomarkers for multi-drug tuberculosis based on data-independent acquisition and targeted proteomics | Groups of participants (healthy control, multidrug-resistant, drug-sensitive)<br><br>Using liquid phase separation and mass spectrometry. Chromatography using a nanolitre flow HPLC system (Easy nLC-1200).<br><br>Data-independent acquisition (DIA) technology is used for vast screening, qualitative, and quantitative analysis of a wide nest of samples.<br><br>Parallel reaction monitoring (PRM) used to verify the identified diverse proteins |
| **Shi et al (2019)** | Plasma indoleamine 2,3-dioxygenase activity as a potential biomarker for early diagnosis of multidrug-resistant tuberculosis in tuberculosis patients | Groups of participants (healthy control, drug-sensitive, multidrug-resistant, lung cancer)<br><br>Using high performance liquid chromatograpy-mass spectrometry (LC-MS/MS) |
| **Yang et al (2019)** | Significance of the differential peptidome in multidrug-resistant tuberculosis | Groups of participants (healthy control, multidrug-resistant, drug-sensitive)<br><br>Differently expressed peptides were analyzed using liquid chromatography- mass spectrometry (LC-MS/MS) and their potential significance was analyzed using ingenuity pathway analysis (IPA) |

Peptidoglycan recognition protein 2 (PGLYRP2) is known in mammals as direct antibacterial and considered as amidase by hydrolyzes bacterial cell wall.[28-30] PGLYRP2 roles in innate immune signaling are changing the recognition of peptidoglycan (PGN) via NOD1/NOD2 receptors.[28,30] The PGLYRP2 plays the important role for host defense toward bacterial lung infection by promoting macrophage activation synergized with TLR2 and TLR4.[30] It also regulates the neutrophils recruitment after respiratory infection. This study found that PGLYRP2 level was more markedly upregulated in the MDR-TB group than DS-TB group. This showed that its increasing related to individual's immune defense response.[28,31]

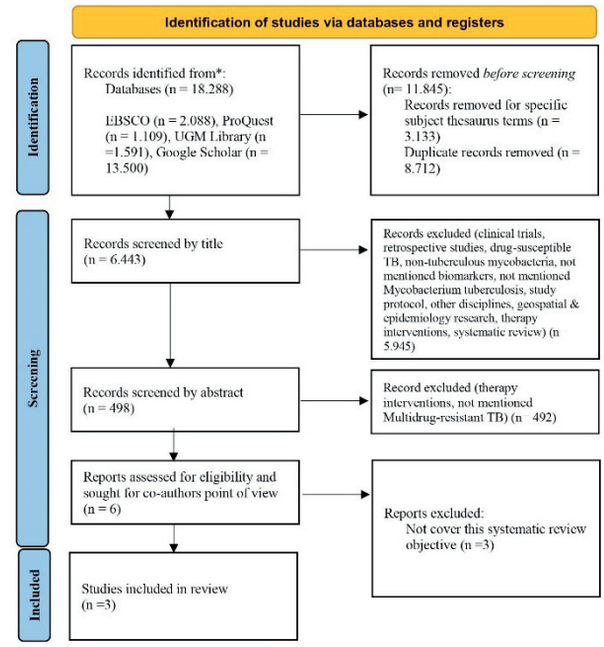


**Figure 1.** PRISMA diagram of the article selection procedure for related articles published between January 2016 and December 2021[29]

Fibrinogen alpha chain (FGA) is a variant of coagulation factor fibrinogen, as component of blood clot, which has molecular mass 420 kDa.[32] This study found that level of fibrinogen in MDR-TB group markedly increased rather than in healthy group. On the other hand, fibrinogen level in MDR-TB group was higher than DS-TB group. This may lead to indication of activated fibrinolytic system due to TB infection progression. Thus, FGA could be used to determine the severity of TB infection.[28,32]

Based on ROC curve analysis and multivariate logistic regression, sensitivity and specificity of sCD14 being biomarkers for MDR-TB and distinguished with healthy individual are 80% and 90%, respectively. Sensitivity and discussion should explore the significance of the results of the specificity of PGLYRP2 to detect MDR-TB

between healthy individual are 60% and 75%. Meanwhile FGA sensitivity and specificity are 75% and 95%. A combination of sCD14, PGLYRP2, and FGA were bringing a diagnostic design with a higher sensitivity (94,7%) and specificity (80%) than the design of a single protein.[28]

**Indoleamine 2,3-dioxygenase (IDO)**

Indoleamine 2,3-dioxygenase (IDO) is a derivate of tryptophan 2,3-dioxygenase (TDO), an intracellular, non-secreted enzyme that induces the tryptophan (Trp) to kynurenine (Kyn) degradation.[33,34] IDO activity is recognized to promote escalating mycobacteria burden and persistence in chronic infection due to its role to suppress CD4+T cell proliferation, promote the differentiation of CD4+CD25+Foxp3+ regulatory T cells (Treg) cells from naive CD4+ T cells, and instigate antigen-presenting cells (APC).[33–35] Higher IDO activity toward the MDR-TB group than in the DS-TB group and its correlation to lung cavity lesions in TB patients were observed. It makes MDR-TB patients present a decreased CD4+IFN-+T cell response and over-induced Treg activation. The cutoff for serum IDO activity in MDR-TB is 46.58 M/mM, with a sensitivity of 87.50%, specificity of 72.22%, and positive predictive value (PPV) of 73.68%. In this study, plasma Kyn and Trp – as two products of IDO metabolic pathway – were measured in MDR-TB patients and substantial differences between MDR-TB and DS-TB patients were observed continuously.[34,36] The IDO activity was also discovered to have a sturdy positive association with lung cavity pervasiveness and size. As a result, it can be concluded that MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) are more likely to be associated with thicker walls and larger cavities. Hereinafter, the more extensive MTB demolition in the patients' lung parenchymal is associated with elevated plasma IDO activity, which might relate to immense cavity lung lesions (cavity prevalence and cavity size). This ensues a significantly higher risk of MDR-TB development and greater pathogen transmission. This study showed that IDO activity plasma can be utilized as a potential biomarker for early MDR-TB identification.[34–36]

**Complement Receptor 2 (CR2) Peptide**

This study identified 40 expressed peptides from collected blood samples of enrolled patients. It found that there were four important differentially expressed peptides in multidrug-resistant tuberculosis: F2, CR2, COL5A2, and ITH4. Cell membrane protein CR2 or CD21 as peptide plays a role as promoting B lymphocyte responses and links the innate and adaptive immune response.[37,38]

Despite those four peptides, CR2 was the main focus due to its association with IL-6, one of the important proinflammatory factors that promote local inflammation.[38,39] After induction of CR2 peptide in a dose-dependent manner, the expression level of IL-6 was decreased significantly. It might be because of CR2 peptide regulating the macrophages proinflammatory cytokines secretion to decrease the local inflammation of the immune response. Through this mechanism, CR2 facilitates immune response to kill the *Mycobacteria tuberculosis*.[37–40]

## SUMMARY

sCD14, PGLYRP2, and FGA may have potential as a diagnostic biomarker for MDR-TB when combined. It showed sensitivity and specificity as 94.7% and 80%, respectively. Otherwise, Indoleamine 2,3- dioxygenase (IDO) and Complement Receptor 2 (CR2) peptide are also strong candidates for MDR-TB biomarkers due to their important role as the pathogenesis marker of MDR-TB. There is a need for further research to investigate those immune response products and their role to eliminate infection of *Mycobacterium tuberculosis* directly.

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## CONFLICT OF INTEREST

The authors declare there is no competing interest.

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**Review Article**

# Human Norovirus Molecular Analysis and Development of Norovirus Vaccine

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***ABSTRACT***

*The most common organism of acute viral gastroenteritis is norovirus, which accounts for roughly 20% of all occurrences of acute gastroenteritis globally. The virus kills over 200,000 children each year and is the leading cause of childhood diarrhea in the rotavirus-vaccinated population. This study aims to review available studies regarding the information on the genogroup norovirus in humans, development of norovirus vaccines, and effectiveness of norovirus vaccines. A systematic review using Science Direct, PubMed, and Scopus databases to identify eligible case studies. The search was conducted in September-October 2021. The quality of the included literature used checklists from the Critical Appraisal Skills Program (CASP). All of the six selected studies with populations given RT-PCR intervention showed positive for norovirus infection. The most predominant genogroups in humans are GI and GII. As for the research results of the two selected studies on norovirus vaccine, namely the human phase 2 trial containing two Virus-Like Particles (VLP) genotypes, one study showed efficacy at 18-49 one study at ≥ 60 years of age. This study analysis uses Takeda bivalent vaccine. The vaccine includes norovirus antigens of the GI and GII genogroups, intending to expand its protective immune potential. GI, GII, and GIV genogroups are prevalent in humans. VLP that contains GI.I and consensus GII.4c have been created as the NoV vaccine, providing significant efficacy. Very likely because they contain GI dan GII antigens, which are the genogroups that infect humans the most. Patients given a placebo developed acute gastroenteritis due to norovirus GII.2, indicating a genotype cross-reactivity.*

***Keywords:*** *norovirus, genogroups, vaccine, human, systematic*

***ABSTRAK***

*Norovirus adalah agen etiologi paling umum dari gastroenteritis akut yang menyebabkan sekitar 20% dari seluruh kasus gastroenteritis akut yang terjadi secara global. Virus ini menyebabkan sekitar 200.000 kematian anak setiap tahun, dan sekarang menjadi penyebab paling umum dari diare anak pada populasi yang divaksinasi rotavirus. Tujuan penelitian ini adalah untuk meninjau studi yang tersedia mengenai informasi tentang genogroup norovirus pada manusia, pengembangan vaksin norovirus, dan efektivitas vaksin norovirus. Systematic review menggunakan database Science Direct, PubmMd dan Scopus untuk mengidentifikasi studi kasus yang memenuhi kriteria. Pencarian dilakukan pada bulan September-Oktober 2021. Kualitas literatur yang disertakan dinilai menggunakan checklist dari Critical Appraisal Skills Program (CASP). Keenam studi terpilih dengan populasi yang diberikan intervensi RT-PCR menunjukkan positif terinfeksi norovirus. Genogroup yang paling dominan pada manusia adalah GI dan GII. Adapun hasil penelitian dari dua studi terpilih pada vaksin norovirus, yaitu uji coba fase 2 pada manusia yang mengandung dua genotipe Virus-Like Particles (VLP), satu studi menunjukkan efikasi pada usia 18-49 tahun dan satu studi pada usia ≥ 60 tahun. Analisis studi ini menggunakan Takeda bivalent vaccine. Vaksin ini mengandung antigen*

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*norovirus genogroup GI dan GII, yang bertujuan untuk memperluas potensi imun protektifnya. Genogroup GI, GII, dan GIV banyak terdapat pada manusia. VLP yang mengandung GI.I dan konsensus GII.4c telah dibuat sebagai vaksin NoV dan memberikan efikasi yang signifikan. Hal ini sangat memungkinkan karena mengandung antigen GI dan GII yang merupakan genogroup yang paling banyak menginfeksi manusia. Pasien yang diberi plasebo mengalami gastroenteritis akut akibat norovirus GII.2, yang menunjukkan adanya reaktivitas silang antar genotype.*

***Kata kunci**: norovirus, genogroup, vaksin, manusia, systematic*

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## INTRODUCTION

Norovirus is a ribonucleic acid virus (RNA) discovered in 1970 with 27 nm diameter, single-strand, and no veil. They belong to the family Caliciviridae.[1] Norovirus is a virus that may cause outbreaks of severe gastroenteritis with the main symptom of diarrhea, which appeared after the Rotavirus vaccine was discovered. Norovirus was found positive in 64 (19%) samples of 340 stools of children with a mean age of 11–12 months (11.75 months). It was generally found in patients suffering from diarrhea under 24 months (95%), with 64% of sufferers male in a study in Indonesia. Infection from norovirus is most common in November, followed by May and April in 2020 because, in that month, it is a rainy season in Indonesia. Important factors that play a role in transmitting norovirus are raining. Rainwater changes the norovirus viral load, and thus, norovirus transmission is easier.[2] Clinical attributes of norovirus infection consist of asymptomatic and symptomatic infections.[3] Clinical symptoms of infection are fever (72%), bloating (59%), vomiting (66%), abdominal colic (34%), anal fistula (27%), seizures (8%), and abdominal distension (16%).[2] Natural infection increases in children during the first two years of life.[4]

Noroviruses are varied in genetic and antigenic properties.[5] One of the epidemics caused by norovirus was the outbreak in Korea in 2013.[6] Kim[6] performed a study and found that of the 230 genotyped norovirus strains, GII.4 (77.3%) was the most prevalent capsid genotypes, followed by GII.3 (6.1%) and GII.13 (3.9%). According to a meta-analysis study, the global prevalence of asymptomatic norovirus is estimated to be 7%, with Africa, Mesoamerica, and South America having a high incidence (11-15%). However, Europe and North America are still having a low prevalence.[7]

People of all ages can be infected with norovirus, with the elderly and youngsters being most vulnerable.[8] Norovirus is the second most frequent source of death by diarrhea for children under the age of five across the region of the World Health Organization.[9] Norovirus outbreaks are prevalent in nursing homes, daycare facilities, and hospitals.[10] Norovirus is highly contagious and pervasive.[11] Various factors that could affect the increase of norovirus transmission are the minimal amount of inoculum required to cause infection (100 particles of the viral agents), prolonged discharge of the viral agent, and its survivability in the environment.[12] This virus plays a significant role in the food-borne epidemic.[13] Efforts are currently underway to develop an effective norovirus vaccine using virus-like particles (VLP), which is regarded as a promising approach to administering cases of norovirus infection.[10] Identification from the study of VLP bivalent vaccine usage on animals and human suggest that multivalent vaccination may be an effective strategy for inducing a broadly neutralizing antibody protective against challenge with the latest and heterologous norovirus strain.[14]

An efficient norovirus vaccine can lower direct influence on gastroenteritis and have indirect socioeconomic costs. The relationship between protective immunity and norovirus infection must be further explored to permit more acceptability and efficacy of norovirus vaccine candidates in humans.[8] Therefore, this study was

conducted to obtain information related to the molecular analysis of norovirus in humans and the development of the norovirus vaccine.

## METHODS

The method used in this study is a systematic review, which focuses on searching comprehensive and detailed data on several relevant works of literature. It aims to reduce biased information by identifying, assessing, and synthesizing all studies relevant to related topics.[15]

The following criteria are used to consider the study for this review: literature that discusses genogroup of norovirus and that of norovirus vaccine, published in 2017-2021, types of literature research articles, and Randomized Controlled Trial (RCT) with full text in English. While the exclusion criteria in this review are works of literature on norovirus genogroup other than in humans and those on norovirus vaccines tested besides humans.

### Finding Strategy

Literature was carried out using the Boolean Operator in OR for literature search with alternative keywords. Thus, broader literature coverage and AND for a complete literature search keyword. The keywords used are entered together in the database using the advanced search. The literature search strategy is carried out with the following two keywords of (Norovirus OR NoV OR Norwalk like viruses) AND Gastroenteritis AND Human AND (Molecular OR RT-PCR) and (Norovirus OR NoV OR Norwalk like viruses) AND Gastroenteritis AND Vaccine AND(Efficacy OR immunogenicity).

### Study Selection

Articles considered potentially eligible for inclusion criteria are selected based on the abstract with PICO criteria. It consists of norovirus patients as a population, molecular development in detection with RT-PCR and the provision of norovirus vaccines as interventions, norovirus identification with analysis (sequencing, phylogenetic analysis, RNA extraction) and placebo administration as a comparison, norovirus genogroup and vaccine effectiveness norovirus as an outcome. Then assessed for quality, literature quality was assessed using the Critical Appraisal Skills Program (CASP). The collected data is then managed by applying the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA). Fundamental literature research obtained will be carried out for Identification, Screening, Eligibility, and Include.

### Data Extraction

This data extraction is done by transferring important information from the literature figures selected into the data collection form. The data are modified from Cochrane (The Cochrane Library). The Cochrane data collection form contains the identity, characteristics, methods, and research results to make it easier for researchers to analyze the writing reviewed for further presentation in a summary table, thereby making it easier for writers and readers to understand the results.

## RESULTS AND DISCUSSION

### Norovirus in Humans

The search results acquired 3,114 articles from Science Direct, 662 from PubMed, and 3,237 from Scopus. The strategy used to filter research articles is with Boolean Logic with the keyword. Furthermore, it was screened using an advanced filter, and the results of the obtained screening of 1,256 works of literature with 17 duplicated literature excluded so that 1,239 works of literature are obtained. The literature was screened by reading titles and abstracts to find 1,228 pieces of literature that do not meet the PICO and sample criteria. After screening, 11 appropriate pieces of literature were obtained to be studied in this systematic review. After further study throughout the literature, five studies were excluded because: Not only focusing on norovirus and discussing other enteric viruses (n=3), discussing other norovirus strains besides GI, GII, and GIV (n=2). So that the results of the literature screening are six pieces of literature that meet the criteria to be studied in this systematic review (Figure 1).

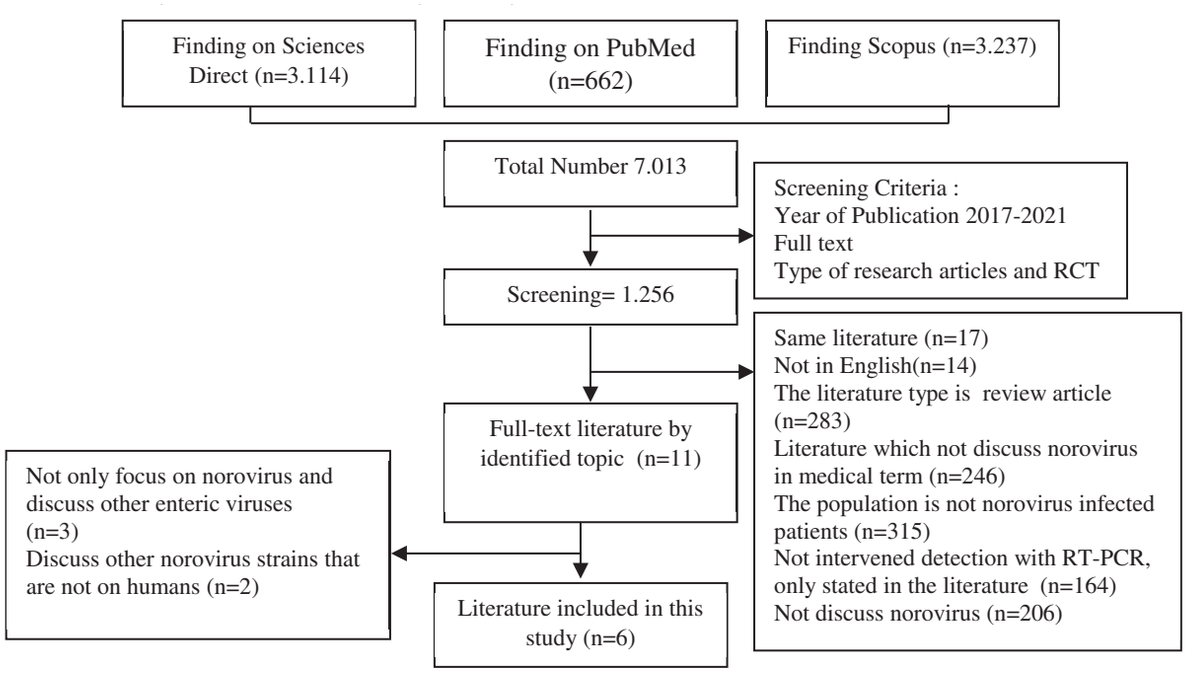


**Figure 1.** Diagram of Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Molecular Norovirus

**Table 1.** Analysis of Molecular Norovirus

| No. | Author | Title | Background | Methods | Time | Participants | Inclusion Criteria | Exclusion Criteria | Variables | Variables Bound | Research Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Toren et al., 2021[16] | Risk factors for norovirus infection in healthcare workers during nosocomial outbreaks: a cross-sectional study, | Gothenburg, Sweden | Norovirus detection by GII specific RT-PCR with a cross-sectional study | January-April 2012 | 308 participants | Health workers in the ward infected with norovirus at Sahlgrenska University Hospital | Cleaners are not employed by the hospital | Fecal samples | Strains of norovirus | A total of 26 out of 129 patients were positive for GII norovirus |
| 2 | John et al., 2021[17] | High proportion of norovirus infection and predominance of GII.3[P12] genotype among the children younger than 5 in Sabah, Malaysian Borneo | Sabah, Malaysia | RT-PCR assay with amplification of C of the capsid region | January 2018–March 2019 | 299 participants | Pediatric patient under 5 years old with acute gastroenteritis admitted to hospital Sabah, Malaysia | Children Aged >5 years old, not suffered diarrhea or fever | Fecal samples | Strains of norovirus | A total of 17.7% of patients were positive for norovirus infection, the majority were caused by the genotypes GII (71,7%), then GI (24,5%), and the combination of GII and GI (3,8%) |
| 3 | Cao et al., 2021[18] | Epidemiology of norovirus gastroenteritis in hospitalized children under five years old in western China, 2015-2019 | Chengdu, China | RT-PCR assay with nucleic acid norovirus kit using Applied Biosystems 7500 | 2015-2019 | 1181 Participants | Pediatric patients under 5 years old with acute gastroenteritis who are hospitalized at Chengdu Hospital, China | Children Aged >5 years old, did not experience Acute gastroenteritis | Fecal samples | Strains of norovirus | 20% of patients were infected with norovirus. Most of the samples came from genotypes GII, id est GII.4 Sydney 2012 and GII.3 |

| No. | Author | Title | Background | Methods | Time | Participants | Inclusion Criteria | Exclusion Criteria | Variables | Variables Bound | Research Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Utsumi et al., 2021[20] | Molecular epidemiology and genetic diversity of norovirus infection in children hospitalized with acute gastroenteritis in East Java, Indonesia in 2015-2019 | East Java, Indonesia | Conventional TaqMan RT-PCR based uses Applied Biosystems 7300 | June 2015-July 2019 | 966 participants | Pediatric patients aged 1-191 months with acute gastroenteritis that hospitalized in the East Java hospital region, Indonesia | Children aged>191 months, No AGE | Fecal samples | Strains of norovirus | A total of 12.3% of samples were detected as positive for norovirus. The predominant genotypes in each year were GII.13 in 2015, GII.4 Sydney in 2016, GII.3 in 2017, and GII.4 Sydney in 2018 |
| 5 | Shen et al., 2020[21] | Molecular epidemiology of norovirus associated with acute gastroenteritis in Taizhou, China: A retrospective study | Taizhou, China | Multiplex RT-PCR with the Applied Biosystems 7500 using AgPath-ID One step RT-PCR kit | January 2016-December 2017 | 1464 participants | Patient of acute gastroenteric, episodic diarrhea and vomiting is in emergency hospital that handle diarrhea | No AGE | Fecal samples | Strains of norovirus | 9.49% of samples of patients with acute gastroenteritis were positive for norovirus. GII was the main genotype. A total of 12 genotypes and seven recombinant strains were found in the study. |
| 6 | Bonura et al., 2021[19] | Recombinant GII.P16 genotype challenges RT-PCR based typing in region A of norovirus genome | Palermo, Italy | QIAamp Viral RNA extraction kit and RT-PCR assay using two sets of modified GII primers (deg 1 and deg 2) | January 2016–December 2019 | 2194 participants | Children under 5 years old being treated for acute gastroenteritis at the children hospital in Palermo, Italy | No AGE Children aged>5 years old | RNA extracted from fecal samples | Strains of norovirus | Norovirus GII identified the most between 2016-2019 were GII.2 and GII.4 Sydney |

## The Study Characteristics of Molecular Norovirus

Studies used in this literature review came from five different regions, each from Sweden, Malaysia, China, Indonesia, and Italy. As shown in Table 1, the population used in the study involved health workers in hospitals in one study[16], pediatric patients aged under five years with acute gastroenteritis in three studies[17–19], children were suffering from severe gastroenteritis in private and government hospitals in Surabaya, East Java in one study[20], acute gastroenteritis patients in southeast China in one study.[21] The intervention used in six references is rectal samples detected for norovirus by specific RT-PCR[16], RT-PCR assay with amplification of region C[17], RT-PCR assay with norovirus nucleic acid kit using Applied Biosystems 7500[18], RT-PCR assay with QIAamp Viral RNA kit[19], Taqman test based on conventional RT-PCR using the Applied Biosystems 7300 system[20], Multiplex RT-PCR assay with Applied Biosystems 7500 using AgPath-ID One step kit.[21] Two studies stated that norovirus-positive patients had GII norovirus, two studies with GII and GI genotypes, and two studies with GII, GI, combination GII, and GI genotypes as shown in Table 1.

Individual contact is strongly associated with norovirus infection. Health workers in large hospitals, especially psychiatric wards, are highly susceptible to norovirus infection. The main symptom of health workers infected with norovirus is experiencing acute gastroenteritis, and the most common norovirus strain is GII.4.[16] The risk of norovirus is higher in children under five years old. It is primarily due to the

strain when the capsid and RdRp genotypes are combined. The highest prevalence of the NoV strain was GII.3[P12], followed by GII.6[P7] and GII.17[P17] in Sabah, Malaysia, from 2018 to 2019.[17] Norovirus of strains GII.17 and GII.2, is one of the most prevalent causes of viral gastroenteritis in children under five in China.[18] Children under two years of age tend to be more susceptible to norovirus infection. In Indonesia, GII norovirus is more connected with disorders that require medical care, whereas GI norovirus causes moderate symptoms and does not necessitate hospitalization. Indonesia's norovirus genotypes are genetically varied and similar to those seen in Asia and Europe.[19]

The norovirus genotypes were dominated by GII, and a small proportion is GI and the combination of GI and GII. The most common strains found in Taizhou, China, are GII.P17/GII.17, GII.Pe/GII.4, and GII.P16/GII.2. The percentage of norovirus infection was 9.49% among all acute gastroenteritis patients of all age groups in Taizhou. The incidence of norovirus infection in various age groups is connected to population immunity, public health, and the usage of over-the-counter medications.[20] Norovirus GII was the most dominant genotype infecting acute gastroenteritis patients in Taizhou. The three main norovirus strains that infect patients in the region are GII.P17/GII.17, GII.Pe/GII.4 and GII.P16/GII.2.[20] The molecular examination is quintessential because the identification of norovirus is generally made by identifying the RNA. Most norovirus outbreaks globally are caused by strains GII.4, including a new variety of GII.4 that appears every 2 to 3 years. The plasticity of the norovirus genome requires periodic renewal of the primers used for RT-PCR amplification[21] (Table 1).

**Norovirus Vaccines**

Based on the search results, there are 2,717 articles with potential from Science Direct, 662 from PubMed, and 3,237 from Scopus. Furthermore, it was screened using an advanced. The results from 135 works of literature were obtained. Then, the researchers excluded six duplications of them to obtain 129 works of literature. The literature was re-screened through reading the title and abstract so that two works of literature were obtained that met the criteria to be studied in this systematic review (Figure 2).

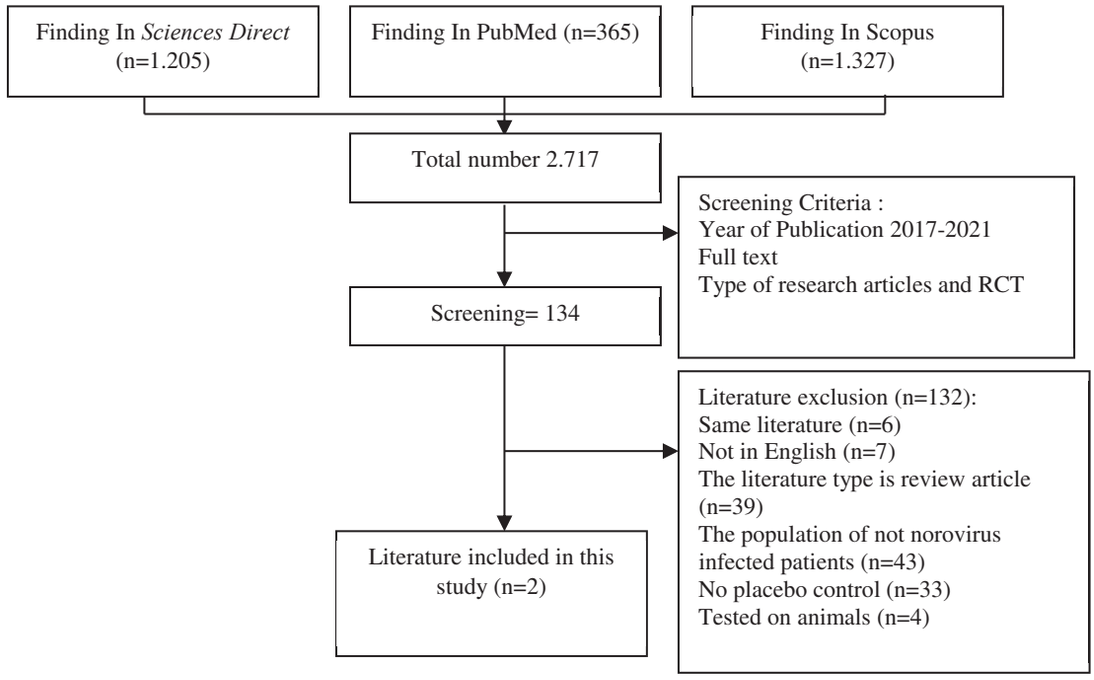


**Figure 2.** Diagram of Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Norovirus

### Study Characteristics of Norovirus Vaccines

The studies used in these two literature reviews are from the United States. The populations used in the literature review are one study using a population of naval soldiers aged 18-49 years[22], and a study using a population of adult subjects aged ≥ 60 years.[23] The two norovirus Takeda vaccine (TAK-214) studies contained 15 µg GI.1 and 50 µg GII.4c VLPs, 0.5 mg Al(OH)3].[22,23] One study showed vaccine effectiveness, indicated by antibodies that can block genotype-specific histo-blood group antigen (HBGA), 80% for GI.I/GII.4 homogenotypes, and 61.8% for any genotypes.[22] There was no safety concern and similar effectiveness of vaccinees over 60 years of age as those of 18-48 years of age[23] (Table 2).

Levels of antibodies that block GI.1 and GII.4c increased in the vaccinated population aged 18-49 years. At the same time, some given a placebo developed acute gastroenteritis due to the GII.2, which means cross-reactivity among genotypes.[22] The adult population over 60 years also showed no worrying conditions when given the vaccine, and the response was similar to the vaccine given to the younger population[23] (Table 2).

**Table 2.** Analysis of the Norovirus Vaccine Study

| No | Author | Title | Backg round | Methods | Time | Participants | Inclusion Criteria | Exclusion Criteria | Variables | Variables Bound | Research Findings |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Sherwo od et al., 2020[22] | Efficacy of an intramuscular bivalent norovirus GI.1/GII.4 virus-like particle vaccine candidate in the healthy US adults | Illinois, United States | Randomized controlled phase 2b trial | June 2016 – June 2018 | 4,712 participants: 2,355 vaccinated and 2,357 saline placebo | Soldier Navy United States in Illinois aged 18-49 years, healthy | Have comorbid disease | Causative genotypes | Effectiveness and Immunoge nicity to vaccine | Level of GI.1 and GII.4c HBGA-blocking antibodies increased on vaccines and in some placebo AGE cases infected with GII.2. It shows that there is a genotype cross-reactivity |
| 2 | Treanor et al., 2020[23] | A phase 2 study of the bivalent VLP norovirus vaccine candidate in older adults; impact of MPL adjuvant or a second dose | 10 trial centers in the United States | Randomized controlled phase 2 trial | February 2016 – October 2017 | 294 participants | Adult subject healthy one same age as or older than 60 years with BMI <35 kg/m2 | Hypersensitivity to vaccine, showing the presence of fever or infection, clinical and mental disorders in immunosup pressive condition | Age strata 60-74, 75-84, ≥ 85 years of age; second vaccination; MPL (monophos phoryl lipid A, an adjuvant) | Immunoge nicity to vaccine | Adults over 60 years old do not present worrisome conditions when vaccinated and respond similarly to that of younger vaccine, and not affected by second vaccination and MPL |

## Discussion

### Molecular Norovirus in Humans

Norovirus is a positive-sense, single-stranded, non-enveloped RNA virus. The norovirus genomes evolve rapidly, resulting in a wide range of genotypes. Noroviruses are now divided into ten genogroups (GI-GX) based on the variations in ORF2, which encodes the VP1 protein. Human infections are caused by the genogroups GI, GII, and GIV. This genogroup was further subdivided into nine GI, 27 GII, and two GIV genotypes. Since recombination in the norovirus genome frequently occurs at the ORF1-ORF2 junction, each genotype consists of those of capsid and polymerase gene, h currently of a polymerase (RdRp) and a capsid (VP1). Based on the RdRp

gene sequence, GI and GII viruses are presently divided into 14 and 37 P-genotypes, respectively. The norovirus capsid consists of 90 capsid protein dimers, forming a shell of 90 protruding arch-like dimers.[19]

Different types of cells in the human gut are involved in norovirus infection. The human gut's predominant cell is a single layer of intestinal epithelial cells (enterocytes), including many immune cells. According to multiple studies, norovirus infects and replicates immune cells such as dendritic cells, B cells, and macrophages. The mechanism is that it enters the host through M cells, which lack microvilli and do not secrete mucous, allowing norovirus to enter the host, attack immune cells, and cause inflammation. The median duration between viral inoculation and clinical manifestations is 1-2 days, and norovirus symptoms usually clear up within 1-3 days. In some cases, symptoms may disappear, and the virus can be excreted in the stool for a prolonged period of up to 60 days.[24]

NoV, specifically genotypes GII.4, evolves quickly through the mutation and recombination events, resulting in the recurrent emergence of new antigenic variants impacted in the global outbreak of acute gastroenteritis.[16] According to Bonura et al.[19], the majority of norovirus outbreaks and sporadic cases worldwide are caused by norovirus GII.4, with new GII.4 variants appearing every 2 to 3 years. The GII.4 variant results from antigenic drift and recombination mechanisms, as evidenced by GII.4 strains epidemics in 2009 and 2012. Additional recombinant norovirus strains have emerged in recent years, particularly at the ORF1-ORF2 junction, including two different GII.4 Sydney 2012 recombination viruses, GII.4 Sydney 2012[P4 New Orleans] and GII.4 Sydney 2012[P16], as well as strains GII.2[P16], GII.3[P12], and GII.3[P16].

Phylogenetic analysis revealed that the Taizhou strains GII.P16/GII.2 shared an evolutionary pattern with the strains discovered in the United States and Japan in 2016.[20] The GII.P16/GII.2 recombination genotypes, previously reported infrequently in China, were first observed in Wuhan province in 2010. This uncommon variant of GII.P16/GII.2 caused a rapid increment in sporadic AGE patient populations in Europe and Asia during the winter of 2016–2017. It can emerge as a widespread strain that can trigger an epidemic or pandemic.

**Norovirus Vaccine**

The development of norovirus vaccines is a priority for both public health and economic benefits. However, there are difficulties in vaccine development due to the complex nature of norovirus, human immune response, viral culture, and limited animal models for vaccine testing. Several vaccines are presently in pre-clinical progress, one of which has finished phase II elderly clinical study. Because of viral evolution, research has focused on multivalent vaccines similar to influenza vaccines, namely Virus-Like Particles (VLP).[23] VLP NoV is a structure that resembles the original virus organization and conformation but lacks the viral genome, potentially resulting in safer and less expensive vaccine candidates.[25]

Takeda vaccine has developed a bivalent intramuscular norovirus vaccine candidate containing two VLP genotypes, GI.1 and consensus genotypes GII.4c synthesized from three variants, GII.4-2006a (Yerseke), 2006b (Den Haag), and 2002. (Houston), intended to provide broad cross-reactivity against various GII.4 strains. Clinical trials have shown that the TAK-214 candidate formulation is safe, well-tolerated, and immunogenic in healthy adults. The ideal adult TAK-214 regimen is a single intramuscular dosage based on the developmentof inhibitory antibodies against Histo-Blood Group Antigen (HBGA), which are expected to correlate with norovirus illness prevention.[22]

Effective norovirus vaccinations for all age ranges are required to lower the worldwide illness burden of the extremely contagious AGE norovirus. Sherwood et al.[22] found that of the 48 cases of moderate or severe AGE norovirus, 29 cases receiving placebo and 19 cases receiving the vaccine, the causative genotypes are GI.1 (n = 1), G1.7a (n = 1), GII.2 (n = 39) and GII.4 (n = 7). Due any to norovirus genotypes, 26 placebo and 10 vaccination groups showed 61.8%

(95.01 % CI, 20.8 to 81.6; p = 0.0097) vaccine effectiveness. While in vaccine genotypes AGE cases, the placebo (five cases) vs. vaccination (one case) group yielded a primary endpoint vaccine effectiveness of 80% (99.99 % CI, 1318.1 to 99.7; p = 0.142).[22] While Treanor et al.[23] showed that this vaccine, with or without MPL, with or without second dose, had similar immune responses. It is expected that the development of the norovirus vaccine will continue to reduce the rate of acute gastroenteritis that has spread throughout the world.

## CONCLUSIONS

The conclusion drawn from the literature review is that the genogroups that infect humans are GI, GII, and GIV, of which GI and GII are the most. The bivalent vaccine being developed, TAK-214, showed significant efficacy to GI.I and GII.4c vaccine antigens and another genotype (GII.2) indicating a genotype cross-reactivity immunity.

Our systematic review is based on many studies and includes several countries worldwide, not only Indonesia. This study provided a more probable estimate of norovirus vaccines worldwide and methods of diagnosing norovirus infection. This study also provided the details of G.I and G.II strains infection in humans. The limitation of this study is the lack of articles about the norovirus vaccine in Indonesia. We could not assess several studies, and their exclusion may have biased our results.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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***Original Article***

# The Longevity of *Aedes aegypti* Larvae in Several Water Sources in Surabaya

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***ABSTRACT***

*Aedes aegypti transmits the dengue virus that causes Dengue Viring the high number of DVI cases is the existing breeding places of Ae. aegypti. The water sources used by the community and the surrounding environment are essential media for living Ae. aegypti larvae. This recent study aimed to detect the longevity of Ae. aegypti larvae in different water sources in Surabaya and the killing effect of temephos. An analytical observational and experimental study was conducted in August-September 2021. Twenty-instar III Ae. aegypti larvae were put in each 100 ml beaker glass containing different water sources, such as rain, well, mineral, new and used bath water, and antiseptic soapy water. Fungi in water sources were examined. Two groups were set with and without temephos, the final temephos concentration was of 0.00001 ppm. Live Ae. aegypti larvae, pupae, mosquitoes were observed every 24 hours for seven days without feeding. Living larvae were still found on Day 7 in all water sources with and without temephos. There were more larvae live in soapy water without temephos, particularly on Day 2 to Day 6, compared to other water sources either without or with temephos. In contrast, many larvae died in mineral water with temephos. Some larvae turned into pupae, started on Day 1. Pupae and mosquitoes were mostly found in rain water with temephos. Ae. aegypti larvae survived better in soapy water either with or without temephos. Temephos seemed to be effective to kill Ae. aegypti larvae in mineral water, and might induce larvae in turning to pupae and mosquitoes quickly at low concentration.*

***Keywords:*** *Ae. Aegypti, larvae, water sources, Surabaya*

***ABSTRAK***

*Aedes aegypti menularkan virus dengue penyebab Infeksi Virus Dengue. Penyakit ini terjadi tertinggi di Asia dan menempati urutan pertama setiap tahun, termasuk Surabaya, Indonesia. Faktor penyebab tingginya angka kasus IVD adalah keberadaan tempat perkembangbiakan larva Ae. aegypti. Sumber air yang dimanfaatkan oleh masyarakat dan lingkungan sekitar merupakan media yang penting bagi kehidupan larva Ae. aegypti. Penelitian terbaru ini bertujuan untuk mendeteksi keberlangsungan hidup Ae. aegypti di berbagai sumber air di Surabaya dan efek membunuh temefos. Studi observasional analitik dan eksperimental dilakukan pada bulan Agustus-September 2021. Dua puluh instar III Ae. Larva aegypti dimasukkan ke dalam masing-masing gelas beker 100 ml yang berisi sumber air yang berbeda, seperti air hujan, sumur, mineral, air mandi baru dan bekas, dan air sabun antiseptik 0,5 ppm. Jamur dalam sumber air diperiksa. Dua kelompok ditetapkan dengan dan tanpa temefos, dengan konsentrasi temefos akhir 0,00001 ppm. Larva Ae. aegypti yang hidup, pupa, nyamuk diamati setiap 24 jam selama 7 hari tanpa diberi makan.Banyak Larva yang hidup*

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*dalam air sabun tanpa temephos, terutama pada hari ke-2 hingga hari ke-6, dibandingkan dengan sumber air lain baik tanpa maupun dengan temephos. Sebaliknya, banyak larva mati dalam air mineral dengan temephos. Beberapa larva berubah menjadi pupa dimulai pada hari 1. Pupa dan nyamuk banyak ditemukan di air hujan dengan temephos. Larva Ae. aegypti bertahan lebih baik dalam air sabun baik tanpa atau dengan temephos. Temephos efektif untuk membunuh larva Ae. aegypti dalam air mineral, dan dapat menginduksi larva berubah menjadi pupa dan nyamuk dengan cepat pada konsentrasi rendah.*

***Kata kunci:*** *Ae. Aegypti, larva, sumber air, Surabaya*

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## INTRODUCTION

*Aedes aegypti* mosquito is a global vector of human diseases, such as yellow fever, dengue, and Zika through the bite of the adult female mosquito. The size and the success for being a mosquito are determined by environmental conditions during the larval growth phase to pupation.[1] The geographic expansion of *Ae. aegypti* has a significant value that has been causing epidemics in different countries of Africa, the Indian Ocean, Asia, Pacific, Europe, and America despite all the considerable efforts made for their control.[2] Almost all tropical countries are not free from the spread of these viruses' diseases by these mosquito carriers. Especially, as a carrier of the dengue virus, *Ae. aegypti* is the primary vector.[3]

In the Southeast Asia and Western Pacific region, about 1.8 billion people are at risk of contracting the dengue virus. Dengue Fever (DF)/ Dengue Hemorrhagic Fever (DHF) epidemics have been reported in Bhutan, India, Maldives, Bangladesh, and Pakistan, and due to the porous borders with India, Nepal is at high risk of DF/ DHF outbreaks.[4] Dengue Virus Infection (DVI) is a public health problem in Indonesia with a fairly high morbidity and mortality rate, and has the potential to cause Extraordinary Events and can also have an impact on community economic losses.[5]

In 2015, cases of DVI in Surabaya experienced many changes, where there was an increase and decrease in different cases every month.[6,7] In 2019, there were 138,127 DVI cases with an Incidence Rate (IR) of 51.48 per 100,000 populations. This number increased compared to 2018 of 65,602 cases. Deaths due to DVI in 2019 also increased compared to 2018 from 467 to 919 deaths.[8]

The development of the *Ae. aegypti* mosquito is based on its ability to adapt to the environment so that it is possible to overcome disturbances caused by natural phenomena. The ability mentioned is about surviving dry conditions and living without water for several months on the sides of the container walls or to adapt to human intervention, such as eradicating mosquito nests.[9] Reproduction sites of *Ae. aegypti* are defined as any water retention container in which the immature stages of *Ae. aegypti* are found. Usually *Ae. aegypti* oviposition sites are found in artificial containers, such as flower pots, stems or water storage tanks, discarded plastic or metal containers, buckets and tires.[10–12]

Clean water used for daily needs produces domestic liquid waste, like waste water from bathrooms that contains soap (NaOH and KOH/ alkali).[13] In a study, it showed that *Ae. aegypti* eggs grow more quickly in water with soap than clean water. This defines bath soap and waste water as the most chosen and better site in the development of *Ae. aegypti* larvae into adult.[13] Another study reveals that *Ae. aegypti* larvae are able to survive in sewer water that has been remained in a single site till it is clear, which means the *Ae. aegypti* eggs which become mosquitoes are more able to breed in clear water than dirty water.[14] Another previous study stated that the most preferred water reservoir properties for the reproduction of *Ae. aegypti* mosquitoes are

well water sources with the complements such as dark in color, without a lid, unexposed to direct sunlight and without draining during more than a week.[15]

In addition, *Ae. aegypti* larvae are able to live together alongside other microorganisms, such as fungi. Fungi usually can be found growing in the same water site as *Ae. aegypti* larvae[13] and could be served as food for the larvae[14]. However, fungi could also be as a lethal pathogen to these larvae and they have been used to control mosquito vectors.[14,15]

Surabaya is a DVI endemic area, and has various water sources in various circles of society. Therefore, research is needed on some of these water sources in order to pay attention to their effect on the growth of *Ae. aegypti* larvae. Moreover, the effectiveness of water sources as a breeding ground for *Ae. aegypti* larvae have not yet been fully studied. The purpose of this study was to detect the longevity of *Ae. aegypti* larvae in several types of water sources in Surabaya, as well as the effect of using temephos on both types of water sources.

## MATERIALS AND METHODS

### Sample Collection

An analytical observational and experimental study was conducted in Institute of Tropical Disease (ITD) Universitas Airlangga, Surabaya, Indonesia from August-September 2021. The sample in this study is *Ae. aegypti* instar III larvae that were collected from the breeding at the Entomology Laboratory, ITD Universitas Airlangga. These larvae were selected using simple random sampling with a total of 20 individuals for each 100 mL beaker glass (Herma, Germany).

### Type of water sources

Variables in this study were rain water, antiseptic soapy water (Dettol[16] with concentration of 0.5 ppm (mg/L)),well water, mineral water, new and used bath water.

### Temephos Preparation

Evaluation of the positive control in this study on the longevity of *Ae. aegypti* instar III larvae used temephos with a concentration of 0.00001 ppm (mg/L). The usage application of temephos was in accordance with the WHO recommendation using the commercial product Abate® 1G (BASF, Indonesia).[16]

### Fungi examination

Fungi examination of each water source was only carried out once on the first day at the Laboratory of Medical Microbiology Faculty of Medicine Universitas Airlangga. The water sources were homogenized by vortex mixer for 30 seconds. One milliliter of each homogenized water source was put in the Saboroud Dextrose Agar (SDA) medium, and kept at room temperature for seven days. Then, fungi were identified from sample film stained with Lactophenol Cotton Blue under light microscope (Olympus© CX22, Japan) with 400 and 1000 magnifications.

### Bioassay

The bioassay for the longevity of *Ae. aegypti* was performed in 14 of 100 ml beaker glasses, divided into two groups of water type. Each group contained six beaker glasses Each beaker glass was filled with each water type. First group was without temephos, second group was treated with 0.00001 ppm of temephos. Each beaker glass was filled with 20 larvae. The other two glass beakers were used as controls, filled with tap water from the laboratory either with or without temephos.

There were 14 beaker glasses, and the total sample was 280 *Ae. aegypti* instar III larvae. The variables were divided into two groups, then the first group was not mixed with temephos, while the second group was mixed with temephos with a concentration of 0.00001 ppm. Then these water sources were filled one by one in 100 mL beaker glass.

These *Ae. aegypti* larvae were observed every 24 hours for seven days without feeding until one had turned into a pupae or mosquito.

### Statistical Analyzes

The data collected are in the form of numbers and percentages, and will be carried out in an average figure completed with its mean value.

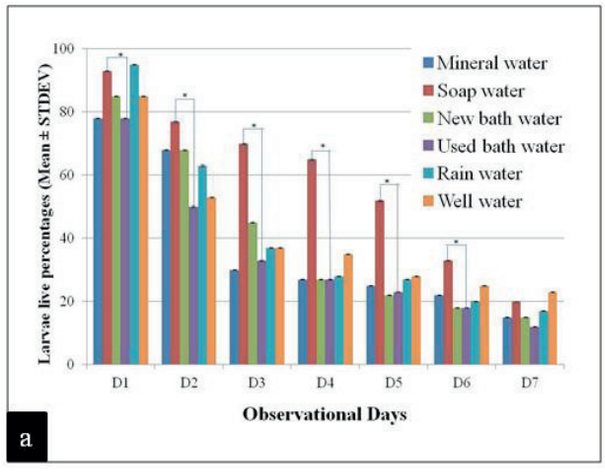
The data variables were also analyzed using the *Chi-square* test with a significant comparison or difference determined by $p<0.05$ value.

### Ethical Clearance

This study has been approved with the license from Medical Research Ethics Commission, Faculty of Medicine, Universitas Airlangga Number 242/EC/KEPK/FKUA/2021.

## RESULTS AND DISCUSSION

This study is the first to be conducted using several different water sources in Surabaya. In addition, this study also used temephos which was mixed in several water sources. Drastically, the average live larvae in mineral water without temephos was reduced significantly on Day 3 (D3) compared to Day 1 (D1), 6/20 vs 16/20 ($p$-value = <0.00001, $p<0.05$, *Chi-square* test). Therefore, the average live larvae in mineral water without temephos seemed to be equal compared to used bath water without temephos since D4 until D7. Interestingly, the average live larvae in soapy water without temephos were decreased little by little per day so that in soapy water without temephos many larvae could still survive (Figure 1a).



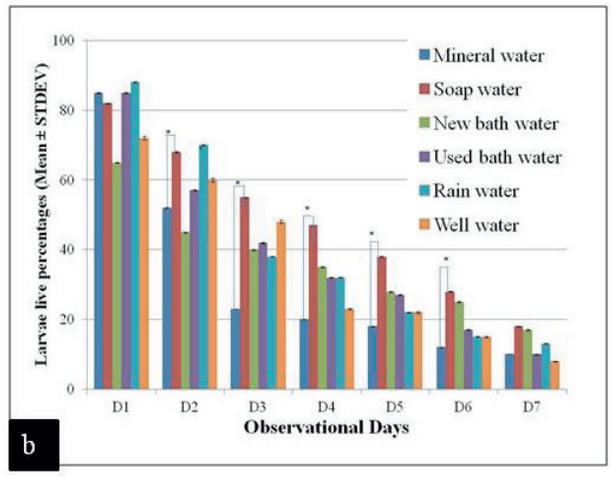




**Figure 1.** Live *Ae. aegypti* larvae inside **a)** water sources without temephos, and **b)** water sources with temephos during seven days of observation. Dark blue bar is mineral water, red bar is soapy water, green bar is new bath water, purple bar is used bath water, blue bar is rain water, and orange bar is well water. *means $p<0.05$, *Chi-square* test Y Axis: Percentage of live larvae ±SD

In mineral water with temephos it was also decreased significantly on D3 compared to D1, 5/20 vs 17/20 ($p$-value = <0.00001, $p<0.05$, *Chi-square* test). Rain water with temephos was the highest among others until D2; however, soapy water with temephos took first place and remained on top until the last day (D7) of the observation (Figure 1b).

In control water, in which the water was taken from the laboratory, it did not demonstrate a significant decrease in the number of live larvae. The results are shown in Figure 2, where on D1 to D4, control water with temephos was higher than without temephos.

However, on D7, the number of live larvae in control water with temephos was lower compared to that of water without temephos, and they were not significantly different (4/20 vs 6/20, $p$-value = <0. 24305, *Chi-square* test).

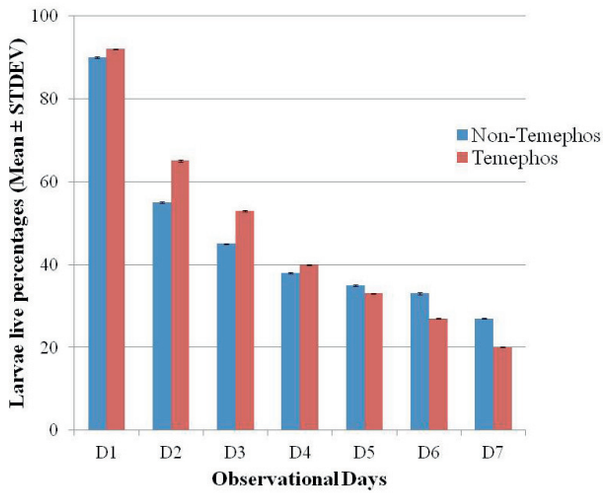



**Figure 2.** Average living *Ae. aegypti* larvae in control water during seven days of observation. Blue bar is control water without temephos, and red bar is control water with temephos

Table 1 shows a calculation of significant differences using *Chi-square* test on the number of live larvae in six different water sources with and without temephos on D1 to D7 of observation. Apparently, only the number of live larvae on D6 and D7 in all water sources without temephos were insignificantly different. Thus, only the number of live larvae in all water sources with temephos on D7 was insignificantly different. The calculation in the control waters was all insignificantly different.

Apart from the larvae that managed to survive, there were also several larvae that achieved in turning to pupae and mosquitoes during the seven days of observation of this study. Afterwards, the results of each water sources demonstrated, even on D1, that there were still some live pupae from mineral water, used bath water and well water without temephos. More pupae were also found in rain water, new bath water, soapy water and mineral water with temephos.

During the seven days of observation, rain water with temephos resulted in thehighest number of pupae. Pupae transformation to adult mosquito took at least two days at average.

However, in temephos water sources, the total number of mosquitoes and pupae were unmatched due to the other two pupae that died during the study. The fact showed that some water sources, such as new bath water and rain water without temephos, as well as used bath water and well water with temephos, did not produce pupae at all (Table 2).

Examinations of fungi on the culture of water samples used in study were also performed. However, no fungi were found in either samples of water, but only the blue color of results was seen on the surface of water culture. This was the absorption of LPCB (Lactophenol Cotton Blue) into the water sample (Figure 3).

**Table 1.** *P*-Value of the Comparison Among the Average of Live *Ae. aegypti* Larvae Inside Water Sources Either with or without Temephos During 7 Days of Observation

| Water Sources | D1 | D2 | D3 | D4 | D5 | D6 | D7 |
|---|---|---|---|---|---|---|---|
| Non-temephos | 0.0008 | 0.0118 | 0.0000 | 0 | 0.0000 | 0.0970 | 0.3549 |
| Temephos | 0.0014 | 0.0023 | 0.0002 | 0.0007 | 0.0255 | 0.0211 | 0.1839 |
| Control | 0.6213 | 0.1489 | 0.2577 | 0.7719 | 0.7655 | 0.3546 | 0.2430 |

*p*<0.05 is significant, *Chi-square* test

**Table 2.** Development of pupae and mosquito during 7 days of observation

| Water sources | n | D0 | | D1 | | D2 | | D3 | | D4 | | D5 | | D6 | | D7 | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | p | m | p | m | p | m | p | m | p | m | p | m | p | m | p | m | p | m |
| a | 60 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| b | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| c | 60 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| d | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| e | 60 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| f | 60 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| a+ | 60 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| b+ | 60 | 0 | 0 | 6 | 0 | 1 | 0 | 0 | 6 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 7 |
| c+ | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| d+ | 60 | 0 | 0 | 4 | 0 | 1 | 1 | 2 | 3 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 9 | 7 |
| e+ | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| f+ | 60 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |

**Abbreviations**: n = larvae total of three times repetition, p = pupae, m = mosquito, a = mineral water, b = new bath water, c = used bath water, d = rain water, e = well water, f = soap water, a+ = mineral water with temephos, b+ = new bath water with temephos, c+ = used bath water with temephos, d+ = rain water with temephos, e+ = well water with temephos, f+ = soap water with temephos

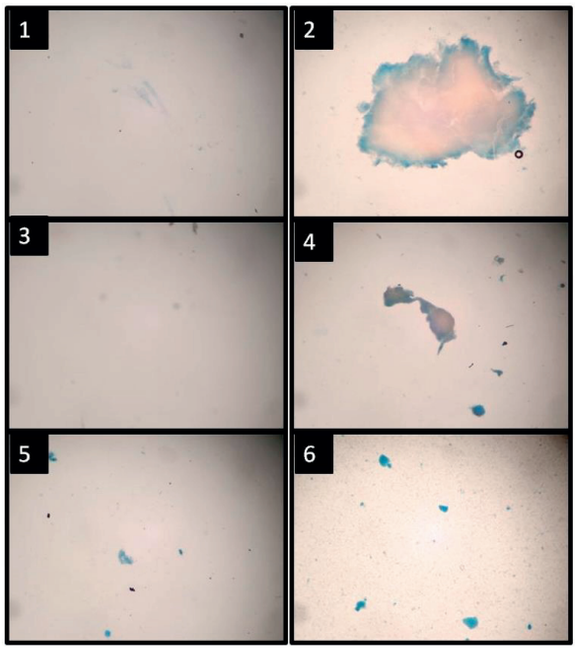



**Figure 3.** No fungi were detected in the water sources; 1) Mineral water, 2) Rain water, 3) Soapy water, 4) Well water, 5) New bath water, and 6) Used bath water

The interesting results of this study showed that larvae were not able to survive in mineral water either with or without temephos. This might explain that the mineral water as a clean water could inhibit the process of larval development to survive and to become pupae. In fact, mineral water identified as microbiologically healthy water had a guarantee of the absence of the most important contamination indicators[17] and categorized its division into macronutrients like calcium, phosphorus, magnesium, sodium and potassium, and micronutrients like cobalt, iron, iodium and copper.[17] In addition, the experiment was conducted in a glass container, which was not the usual breeding place of *Ae. Aegypti.* The natural breeding places of this mosquito are flower pots, stems or water storage tanks, discarded plastic or metal containers, buckets and tires.[10–12,] Moreover, Baharuddin and Rahman[22] found that *Ae. aegypti* larvae were mostly obtained in plastic containers such as plastic barrels and used rubber tires[18]. It suggested that *Ae. aegypti* larvae could not live long inside a clean glass containing mineral water.

On the other hand, soapy water either with or without temephos was very prominent with a high percentage of live larvae. This means that *Ae. aegypti* larvae could still survive better in water mixed with antiseptic soap 0.5 ppm, rather than other types of water. This might be because the soapy water contains sodium palmate, talc, sodium palm kernelate and paraffin liquidum[19] that could provide food for these larvae to survive. Another study stated that soapy water with an equivalent concentration on water pollution in

nature also could become a good breeding place for *Ae. aegypti* larva to survive; however, it only works if the pH of the soapy water is less than 12.8.[20] It is suggested that temephos with a little concentration of 0.00001 ppm did not work effectively in soapy water. Therefore, the water for bathing and water reservoir should be drained.[21]

In control waters, no significant difference was found between water with or without temephos. It seemed that the control waters as media for living larvae were similar condition and the concentration of 0.00001 ppm temephos showed a low efficacy of larvicide.

There was no discovery of fungi in water sources used in this study. This happened because it was possible that the water sites where the water was taken had no prospect to grow fungi. Fungi usually grow in environments that have soil debris, insect remains, or dead leaves and plants.[22]

A study revealed that fungi are used as food and provide nutrients for larval development. Therefore, fungi-mosquitoes associations are able to form a more commensal period in the gut of mosquito with slight or no effect on host survival.[23] An example is the yeast, *Saccharomyces cerevisiae,* which is commonly used to feed the larvae during its developmental phase.[23] The feeding behavior of adult mosquitoes also leads to the formation of adhesions of the fungi in the mosquitoes' hindgut. At least there are four fungi species of the genus *Smittium*, of the order *Harpelellas* that can attach to and increase on various mosquito species' hindgut without affecting larval development or survival.[23]

On the other hand, there have been studies demonstrated the potential usage of fungi as a successful and ecologically safe strategy to control mosquito vectors.[24]

Since few studies reported that the number and diversity of fungi are greater found on the surface water than in groundwater and tap water[25–27], the fungi are possibly contact with adult mosquitoes, some of which fungi are already infused together with chemical insecticides.[28,29] Besides chemical materials, some of the fungi itself are pathogens to mosquitoes and larvae. Fungi species such as *Entomophthora* sp. and *Coelomomyces* sp. are known as obligate pathogens, while other fungi order such as *Eurotiales, Hypocreales* and *Mucorales* are opportunistic pathogens that unfortunately cannot actively invade the mosquito body, but can set up an infection if ingoing through breaches in the cuticle.[23] Other fungal pathogens from water molds such as those in the genera *Lagenidium, Leptolegnia* and *Saprolegnia* are identified as facultative pathogens of mosquitoes, and obviously there are no commensals between these fungi and mosquitoes nor larvae.[23] Thus, there were no fungi in water sources in our study, which showed no fungi effecting into the larva life of *Ae. aegypti* in our study.

The use of temephos with concentration of 0.00001 ppm was applied in this study in order to find out its effect on the larval longevity. Temephos worked very well on killing *Ae. aegypti* larvae in mineral water, and water sources with temephos showed *Ae. aegypti* larvae turned to pupae and adult mosquitoes rapidly. The temephos' concentration of 0.00001 ppm seemed to effectively induce larva development into pupa.

Several studies have shown that the use of temephos could kill *Ae. aegypti* larvae very quickly, because the toxicity of temephos is absorbed into the body of the larvae.[30–32] The absorbed toxin attacks the larvae's central nervous system, causing symptoms such as restlessness, hyperexcitability, tremors, convulsions, and paralysis.[32]

Temephos inhibits cholinesterase enzyme, which causes a disorder in the larva nervous system due to the accumulation of acetylcholine in nerve endings, and this will lead to the larval mortality.[30–33] A study in South Kalimantan showed that the lowest concentration of temephos was 0.005 ppm resulted in 39% of larvae mortality. The highest concentration of 0.030 ppm resulted in 100% of larvae mortality.[34] Comparing to other study, the *Ae. aegypti* larvae were continuously exposed with larvicide such as temephos, over a particular time at the larvicide would make a modification in the larvae genetics and brings resistance to temephos and other larvicides.[35,36]

In this study, the use of temephos was at a concentration of 0.00001 ppm, where this concentration was very small and probably the concentration lacked the scale of larval killing when compared to the study in South Kalimantan. However, if observed from the overall point of view, this very small concentration of temephos could still kill *Ae. aegypti* larvae, particularly in mineral water, and showed the induction of larva development into pupa. Regarding this point, the use of temephos for larvicide should be adequate and in appropriate dose, based on the instruction written on the package and guidelines by Kemenkes RI and WHO.[21,37]

## CONCLUSIONS

*Ae. aegypti* larvae endured better in antiseptic soapy water with concentration of 0.5 ppm either with or without temephos compared to other water sources. Temephos with concentration of 0.00001 ppm was effective to kill *Ae. aegypti* larvae in mineral water, and might induce larval development into pupae and mosquitoes more quickly.

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## CONFLICT OF INTEREST

There are no conflicts of interest between authors in this study.

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**Review Article**

# *Clostridium difficile* Infection (CDI) by Hypervirulent BI/NAP1/027 Strain: a Comprehensive Review of Toxigenicity, Pathogenesis, Risk Factors, and Preventative Measures

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***ABSTRACT***

*Clostridium difficile is an anaerobic bacil gram-positive bacteria, able to form spores and toxin, that is transmitted among humans through the fecal–oral route. Clostridium difficile infection (CDI), a typical nosocomial infection has been contributed to a significant proportion of morbidity and mortality among in-patients with a case-fatality rate of 14% within 30 days after diagnosis. Profound culture and toxin examination for C. difficile are still minimal in many hospitals in various Asian countries. Consequently, C. difficile reports in Asia remain rare. Highly virulent form of C. difficile caused greater fatality and epidemics severity. Elderly age, hospitalization, exposure to antibiotics e.g., cephalosporins, fluoroquinolones, clindamycin, and penicillin contributed as main risk factors. Hypervirulent strain BI/NAP1/027 demonstrated to carry CdtLoc gene locus encodes CD196 ADP-ribosyltransferase (CDT) or known as binary toxin. Virulence factors are TcdA, TcdB, CDTa CDTb in which hypersporulation and mutation of TcD gene by hypervirulent strain led to toxin hyperexpression. Early cases detection, building management team to evaluate patient positive with all C. difficile toxins, hand hygiene improvement, continuation of contact precautions after diarrhea resolution, audit of infection control, and restriction of antimicrobials should be implemented as preventative measures. Focus measures also should emphasize on development of vaccine of C. difficile to boost immune state of elderly people. This review aims to describe severity of disease caused by hypervirulent BI/NAP1/027 C. difficile strain, its mechanism or pathogenesis, risk factors, current treatment options available, along with proposed preventative measures and infection control.*

***Keywords**: Clostridium difficile infection (CDI), hypervirulent strain, BI/NAP1/027*

***ABSTRAK***

*Clostridium difficile adalah bakteri basil gram positif anaerobik, pembentuk spora dan toksin, yang ditularkan di antara manusia melalui rute fekal-oral. Clostridium difficile infection (CDI), sebuah tipikal infeksi nosokomial telah berkontribusi pada proporsi yang signifikan terhadap morbiditas dan mortalitas di antara pasien rawat inap dengan tingkat fatalitas kasus 14% dalam waktu 30 hari setelah diagnosis. Kultur dan pemeriksaan toksin C. difficile masih minim di banyak rumah sakit di berbagai negara Asia. Akibatnya, laporan C. difficile di Asia masih jarang. Epidemi kematian dan keparahan yang lebih besar dari CDI disebabkan oleh C. difficile yang hipervirulen. Faktor risiko utama adalah usia lanjut, rawat inap, paparan antibiotik misalnya sefalosporin, fluoroquinolones, klindamisin, dan penisilin. Strain hipervirulen BI/NAP1/027 terbukti membawa lokus gen CdtLoc yang mengkode CD196 ADP-ribosyltransferase (CDT) atau dikenal sebagai toksin biner. Faktor virulensi yaitu TcdA, TcdB, CDTa CDTb; strain hipervirulen mampu melakukan hipersporulasi dan mutasi gen TcD yang menyebabkan hiperekspresi toksin. Tindakan pencegahan dapat dilakukan dengan deteksi dini kasus, pembentukan tim manajemen untuk mengevaluasi pasien yang positif semua toksin C. difficile, peningkatan kebersihan tangan, kelanjutan tindakan pencegahan kontak setelah resolusi diare, audit pengendalian infeksi, dan pembatasan antimikroba. Fokus upaya juga sebaiknya ditekankan pada pengembangan vaksin C. difficile untuk meningkatkan*

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*status kekebalan pada individu berusia lanjut. Tinjauan ini bertujuan untuk menggambarkan tingkat keparahan penyakit yang disebabkan oleh strain C. difficile BI/NAP1/027 hipervirulen, mekanisme atau patogenesisnya, faktor risiko, pilihan pengobatan yang tersedia, serta tindakan pencegahan dan pengendalian infeksi.*

***Kata kunci:*** *Clostridium difficile infection (CDI), strain hipervirulen, BI/NAP1/027*

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## INTRODUCTION

*Clostridium difficile* infection (CDI) has been known as a typical nosocomial infection and contributes to a significant proportion of morbidity and mortality among in-patients with a case-fatality rate of 14% within 30 days after diagnosis.[1] *C. difficile* gives rise to numerous infections varying from mild diarrhea to pseudomembranous colitis (PMC), mainly in elderly patients with antibiotic treatment. In addition, high healthcare costs related to CDI increase the financial burden of government on health expenditure. It was recorded that half a million infections were attributed to CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009.[2,3] A literature study by Collins et al.[4] found few data of CDI cases. The study found that a study in Japan only reported on the ribotyping result of *C. difficile* without any information on CDI prevalence or incidence in Japan; CDI incidence increased from 1.7/1,000 to 2.7/1,000 adults in Korea, and 17.1/10,000 inpatients in Shanghai were attributed to CDI. Meanwhile, about 44% and 14% of colitis positive patients were positively diagnosed with the *C. difficile* toxin in Philippine and Malaysia, respectively.[4] A more recent study showed that CDI prevalence was 9.2% in Thailand.[5] There are only a few reports about CDI incidence or prevalence in Indonesia. A study reported that there were eight types of *C. difficile* strains presenting in healthy people,[6] while another study showed that the prevalence of *C. difficile* (toxin A) was 1.3% in community and hospital in Jakarta.[7] The last report originated from Central Java showing the prevalence of CDI to be 20.6% by 2017.[8]

Profoundly extensive culture and toxin examination for *C.difficile* are still minimal in many hospitals in various Asian countries. Consequently, *C.difficile* reports in Asia remain rare. In the current study held in Malaysia, assays determining toxin A/B from 175 stool samples collected from patients with antibiotic-associated diarrhea have been performed in tertiary hospital in north-eastern suburb; 24 of them (13.7%) tested positive for toxin, where the age most of infected patients is >50 years.[9-11] However, no ribotyping or any other molecular test have been done in regard to isolates of Malaysian *C. difficile*. Similar to Malaysia, CDI cases reporting in Indonesia is uncommon. It has been found 1.3% test results of stool sample reveal the etiology of diarrhea in Indonesia children was *C. difficile*. Furthermore, only enzyme immunoassay of toxin A was conducted; therefore, the *C. difficile* true prevalence may have been substantially greater. Molecular study of eight isolates collected from Indonesia established five of the results identified as toxinotype VIII and ribotype 017, assembled into epidemic strains of international 017. Two of them are A+B+ toxinotype 0, and one remaining A-B+ isolate was identified as toxinotype XVI binary toxin.[12-14]

Some risk factors including advanced age, antibiotic exposure, and hospitalization are highly associated with CDI.[15] Regulation of antibiotic usage in Asian countries is considered to be poor. There has been a review in Southeast Asian countries which depicted 47% of pneumonia cases as not receiving proper antibiotic whereas 54% of patients with diarrhea were receiving antibiotic unnecessarily, with 40% of under-dose antibiotics prescribed. The advanced age individuals with recent antibiotic treatment are at

the highest risk for CDI as they lack of beneficial gut microbiota and have low immunity due to age and other comorbidities.[16-20] This group is excessively affected and has the highest mortality rate due to CDI with 2% of risk increase every year after 18 years old of age. A report described around one in ten deaths due to CDI in advanced age people in the USA in 2010. There were no data of CDI on advanced age people in Indonesia, which could be due to lack of surveillance on CDI cases followed by limited laboratory facility in the hospital capable of diagnosing CDI. Besides, recurrence (relapse/reinfection) and death cases due to CDI in advanced age people would be higher because of improper treatment.[21]

Severe form of *C. difficile* infection (CDI) is caused by hypervirulent strain identified as type 1 of North American pulsed field, type B1 class from analysis of restriction-endonuclease, ribotype 027 as presented by PCR. Hypervirulent strain leads nationwide CDI outbreaks in European countries, Canada, and the United States (U.S.). First outbreak report of type 027 CDI was in Canada where the worst infected was Quebec in 2005. In the U.S., type 027 CDI affected 38 states. Meanwhile based on European Centre for Disease Prevention and Control, there were infections in 16 countries due to type 027 CDI. Hypervirulent strain of toxinotype III nurture TcdA and TcdB toxin genes, possess deletion of 18-bp in TcdC of toxin regulatory gene, and deletion at area 117. It leads to premature stop codon and frameshift, causing TcdC protein truncation. The rising case of type 027 virulence associated with more excessive toxin production can be attributed to lack control of regulatory TcdC.[19-21]A cohort study estimated that around 40% of CDI cases were community-acquired CDI (CA-CDI). CA-CDI occurs in younger age people, less severe symptoms, shorter hospital stay, lower recurrence rate and no deaths have been reported attributable to CA-CDI. Besides, CDI is also exacerbated by the discovery of hypervirulent strains and antibiotics resistant to quinolones, gatifloxacin instead of levofloxacin.[17-19] The emerging of CA-CDI will be a risk factor for domestic and foreign tourists who visit Bali.

Since 2000, greater fatality and severity epidemics of CDI have been caused by a highly virulent form of *C. difficile*. BI/NAP1/027 strains have spread widely and robustly over past 10 years and have been associated to CDI epidemics. The prevalent ribotypes in the Middle East are 140, 126, 078, 046, 014, 002, and 001, meanwhile the more prevalent ribotypes in Asia are 018, 017, 014, 002, and 001. In North America and Europe, ribotypes 078, 027, 020, 014, and 001 have been the uppermost strains.[22-24] Ribotype 027 has been found to possess reduced susceptibility to chloramphenicol, imipenem, clindamycin, moxifloxacin, rifampicin, and metronidazole. These characteristics implicate to more severe presentation of disease, high morbidity and mortality rates due to antimicrobial resistance juxtaposed to other strains. Spores of ribotype 027 expand more robustly and easily in hospital as they able to resist disinfectants, cleaning, and hospital surroundings. Observational study on patients with diarrhea in Veteran Affairs Medical Center, U.S. demonstrated around 22% of them were positive of BI/NAP1/027 strain.[19-24] This literature review aims to describe severity of disease caused by hypervirulent BI/NAP1/027 *C. difficile* strain, its mechanism or pathogenesis, risk factors, current treatment options available, along with proposed preventative measures and infection control.[22-24]

## *Clostridium difficile* INFECTION (CDI)

*Clostridium difficile* is an anaerobic gram-positive bacillus bacterium, able to form spore and toxin, transmitted in humans by fecal–oral pathway. In the U.S., *C. difficile* is the most frequent nosocomial pathogen reported. A surveillance study of 2011 found 453,000 CDI cases with 29,000 associated deaths, wherein around a quarter of those were community-acquired. Nosocomial *C. difficile* infection quadruples hospitalizations cost causing rise of expenditures by about $1.5 billion in the U.S. yearly. It was recorded that half a million infections were attributed to CDI in the United States in 2011 with an incidence rate of 8.75 cases/1,000 adult admissions in 2009. In Hong

Kong, there were more than fifteen thousand CDI cases from 2006 to 2014 in which most were identified as a nosocomial infection. A nationwide study in Korea revealed CDI total incidence was 2.7 cases/1,000 adult admissions in 2008. CDI is also known for its propensity to recurrence among 35% of patients with antibiotic therapy and more than a half of recurrences of CDI are identified as relapse (relapse or reinfection).[25]

Due to CDI, approximately $1.1 billion are utilized in healthcare cost annually in the USA, while about €3 million is associated with healthcare costs in Europe. Compared to reports from countries across Europe and the USA, the prevalence of CDI in Asia is not fully known. In Korea, survey across 17 tertiary hospitals, from 2004 until 2008, found CDI incidence cases soared from 1.7/1,000 to 2.7/1,000 adults. Community-acquired CDI (CA-CDI) proportion over total CDI cases in a hospital in Busan was 7.1%, meanwhile 59.4% of CDI cases at a Seoul hospital's emergency department were CA-CDI. Based on a comprehensive study in Shanghai, China from March 2007 until April 2008, overall CDI incidence was 17.1/10,000 admissions; mild CDI because of younger mean age (62.8 years) compared to 63% patients were ≥65 years in a comprehensive European study. In addition, a survey across 13 Asia-Pacific countries demonstrated the proportion of CDI associated to healthcare facility was 53.6% and CA-CDI was 16.5%.

CDI case reports in Indonesia remain uncommon. *C. difficile* was identified in 1.3% stool samples of Indonesian children. However these data were not enough to reflect global prevalence in Asia. Furthermore CDI prevalence data of elderly are still unavailable to date.[22-26]

CDI mostly occurs in advanced age people, which is possibly explained by some of the risk factors, including frequent exposure to healthcare, age-related changes in physiology, increasing antibiotics usage, changes in the composition of gut flora, and increased comorbidities. Frequent exposure to healthcare increases the opportunity of contacting with environments contaminated with endospores of *C. difficile* and frequent utilization of antimicrobials. Carriers of *C. difficile*, both asymptomatic and symptomatic, could contain spores on their skin and discard those into the environment. Age-related physiologic changes also increase the risk of CDI, particularly changes in the immune system. The development and recurrence of CDI have been associated with the ability to generate immune responses, and the ability to produce antibodies against toxin may affect the progress of colonization and active infection. Aging is accompanied by immune senescence – a degeneration of the immune system related to advanced age – and it has been associated with a diminishing adaptive immune system.[27-29]

*C. difficile* has the ability to do colonization in large intestine, then releasing exotoxins protein (TcdA, TcdB) leading to colitis in people with risk factors. Figure 1 depicts TcdA and TcdB arbitrate *C. difficile* diarrhea, causing Rho family members' inactivation, Rho GTPases (guanosine triphospatases). This is followed by neutrophilic colitis, colonocyte death, functional loss of intestinal barrier, and death of colonocytes. Expression of clinical CDI disease is exerted by host immune responses and strain of *C. difficile*. A dramatic increase of severe CDI in hospitals was initially reported in the beginning of 2000s. CDC (Centers for Disease Control and Prevention) depicted isolates were group BI of restriction endonuclease, NAP1 (gel electrophoresis of North American), and PCR (polymerase chain reaction) 027; therefore, as BI/NAP1/027. This strain's characteristics are high level resistance of fluoroquinolones, robust production of toxin, efficient rate of sporulation, and significantly high mortality compared to less virulent *C. difficile.*[28,29] BI/NAP1/027 strain firstly originated in North America and Western Europe, but currently it spreads to various settings of hospitals across the globe.[30,31]

Even though hospital-acquired CDI has been the majority, CA-CDI has been increasing significantly and contributes to a third of new CDI cases. CA-CDI happens when onset of disease begins within 12 weeks in individuals who did not stay overnight in hospitals or other healthcare facility. CA-CDI could occur in younger patients, who have unclear antibiotics

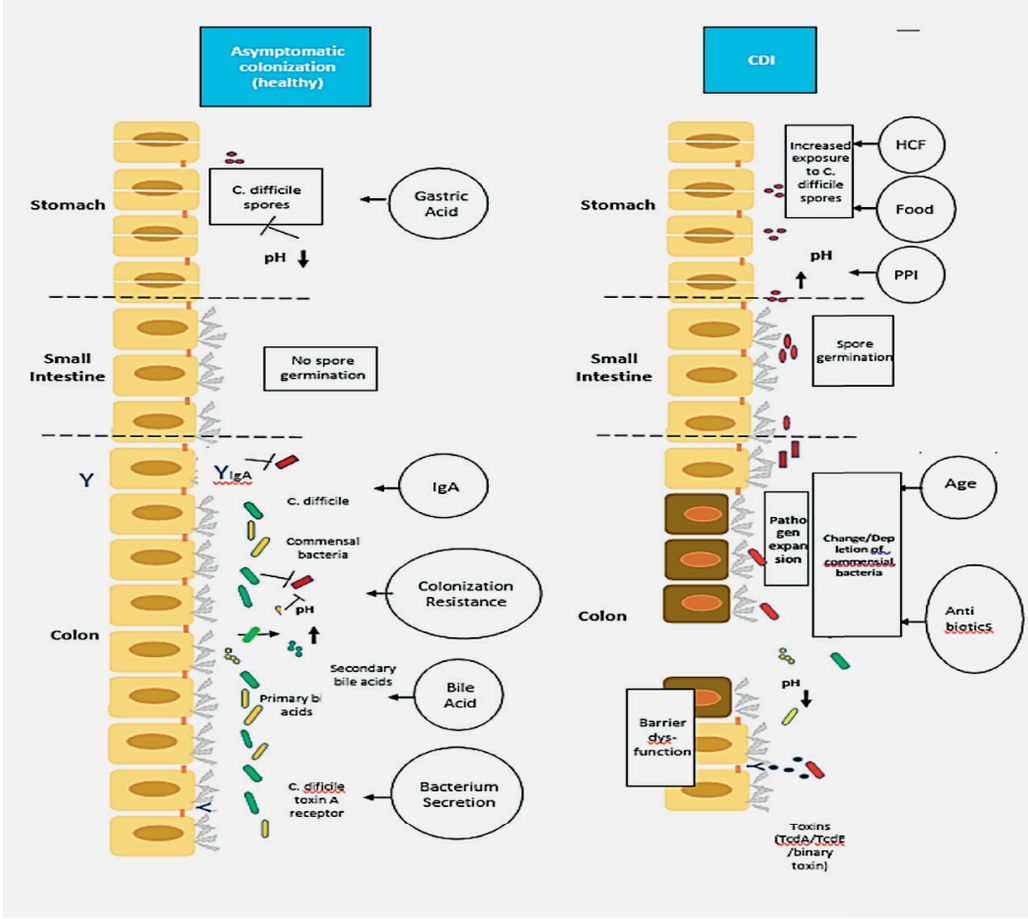


**Figure 1.** Progress from asymptomatic colonization to *C. difficile* infection (CDI)[22-29]

exposure and unknown risk factors. Therefore, CA-CDI acquisition main modes are currently in investigation. CA-CDI associated morbidity and mortality remain lower compared to hospital-acquired CDI. Nonetheless, 40% of CA-CDI patients need hospitalization and relapse rates are similar to HA-CDI.[32] Acid suppression influence to CDI remains unclear. Theoretically, gastric acid suppression allows more vegetative organisms to reach colon. However, *C. difficile* produces spores resistant to acid pH.[33,34]

## HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN RISK FACTORS

Substantial risk factors of CDI by BI/NAP1/027 strain are namely hospitalization, elderly age, and exposure to antibiotics, e.g., cephalosporins and fluoroquinolones. Particular fluoroquinolones identified being risk factors are ciprofloxacin, gatifloxacin, moxifloxacin, and levofloxacin, presumed as consequences of fluoroquinolones-resistance in endemic strain. Almost all antibiotics of cephalosporin class are resistant to all *C. difficile* types and have been incriminated as significant risk factor in hospitals where endemic strain exists, as well as its usage for surgical prophylaxis. Consumption of agents to lower stomach acid production, e.g., proton pump inhibitors (PPI), and type 2 blockers of histamine have been recognized inconsistently as CDI risk factors in hospital with predominance of endemic strain. Besides resistance to current fluoroquinolones, other specific factors of BI/NAP1/027 strain dissemination as well as severe CDI caused by this hypervirulent strain remain speculative and need to be the substance of thorough study or research.[32-34]

Administration to almost all groups of antimicrobials has been delineated to cause CDI, even though cephalosporins, clindamycin,

penicillin, and present fluoroquinolones are notably reported as common culprits. Numerous isolates of *C. difficile* were found containing elements of mobile genetic, markers of antibiotic resistance in chromosomes, and mutations of genetic conferring resistance into rifamycins, chloramphenicol, tetracyclines, streptogramin, lincosamide, macrolide, and fluoroquinolones. Patients also progress to CDI disease following antibiotics therapy which leads to susceptibility of *C. difficile* strain infection. Presumably, CDI occurs due to suppression of normal microbiota in intestine for extended periods after discontinuation of antibiotics; therefore, allowing sustained opportunity for colonization and infection of *C. difficile*. Isolates resistance to clindamycin, erythromycin frequently associated to epidemics and outbreaks. Furthermore, individuals administered by clindamycin possess a remarkable high-rise CDI frequency caused by clindamycin resistant. Resistance is commonly associated with erm(B) presence encoding methyltransferase and ground in Tn5398 conjugative transposon.[35] Current exploration of BI/NAP1/027 isolates has exemplified re-emergence of erythromycin-resistant BI/NAP1/027 in European countries, the U.S., and Canada.

Currently, therapy with fluoroquinolones is identified as BI/NAP1/027 *C. difficile* infection risk factors, and there is a proposed association between therapy with fluoroquinolones and emergence of BI/NAP1/027 strain. Even though all previous isolates of BI/NAP1/027 are susceptible to gatifloxacin, moxifloxacin, and fluoroquinolones, yet resistant to levofloxacin and ciprofloxacin, almost all current isolates were resistant to all fluoroquinolone antibiotics. Inhibition of DNA replication by fluoroquinolones is as a result of its binding to DNA gyrase or topoisomerase II, or topoisomerase IV. Resistance to fluoroquinolones in *C. difficile* is associated with particular mutations in gyrA and gyrB, that encode DNA gyrase. Fluoroquinolones are broad-spectrum antibiotics which act on gram-negative and gram-positive bacteria and are able to decrease normal flora in intestine, hence broad use of fluoroquinolones in hospitals fosters spreading of BI/NAP1/027 *C. difficile* strain.[32-36]

## HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN TOXIGENICITY AND PATHOGENESIS

Hypervirulent strain BI/NAP1/027 is demonstrated to carry CdtLoc gene locus and encodes CD196 ADP-ribosyltransferase (CDT) or known as binary toxin. Hypervirulent *C. difficile* is able to produce TcdA and TcdB, similar with non-027 ribotypes throughout gene locus of PaLoc. CDT was initially isolated by Popoff et al.[37] The toxin contains two distinct toxin components separately, namely CDTa and CDTb. CDTa, ADP-ribosyltransferase enzyme acts on actin modifying which leads to depolymerization and destruction of actin cytoskeleton inside gut; meanwhile CDTb hitches to gut cells and stimulates CDTa uptake. Destruction by CDT accommodates bacteria adherence and surges Toxin A and B uptake.[38-40] Furthermore, hypervirulent strain contains bp frameshift deletion on TcdC gene, nucleotide 117, and functions as negative regulator of Toxin A and Toxin B. TcdC mutation causes toxins hyperexpression. Warny et al.[58] demonstrated BI/NAP1/027 as able to produce 16 times of Toxin A and 23 times of Toxin B approximately compared to control strain. One research postulated increasing sporulation by hypervirulent strain possibly has association with robust CDI spreading. Nevertheless, previous research demonstrated controverted results in regard to disease severity by hypervirulent strain. A retrospective study by Bauer et al.[41] concluded hypervirulent strain BI/NAP1/027 as associated with declined odds of disease severity ratio (OR): 0.35, 95% confidence interval (CI) 0.13 - 0.93) and did not increase mortality in hospitalized patients (OR: 1.02, 95% CI 0.53 - 1.96), or (OR: 1.16, 95% CI 0.36 - 3.77) of recurrence rate. Meanwhile, some other studies (cohort, case-control, and cross-sectional) did not demonstrate worse prognoses compared to other strains as shown in Table 1.[41]

**Table 1**. Virulence factors of hypervirulent BI/NAP1/027 *C. difficile* strain[41]

| Virulence factors | Mechanism |
|---|---|
| TcdA or Enterotoxin A (Toxin A) | Destruction of actin within target cells causes infiltration of neutrophil, inflammation, and epithelial cells necrosis. |
| TcdB or Cytotoxin B (Toxin B) | Destruction of epithelial cells tight junctions causes increasing permeability of vascular, and hemorrhage. |
| CDTa toxin | Modifies the action with ADP-ribosylation leads to depolymerization of actin and damage of cytoskeleton assists bacteria adherence to epithelial cells of gut. |
| CDTb toxin | Facilitates CDTa toxin uptake into epithelial lining of gut. |
| Hypersporulation | Increases bacteria reproduction and spreading. |
| Mutation of TcD gene | Increases assembly of Toxin A and Toxin B by down-regulation of feedback inhibitor necessitate in diminishing toxin production. |

Based on Sirard et al. (2011), even though hypervirulent strain BI/NAP1/027 is able to assemble more toxins, they construct spores in fewer numbers and have not always been associated with severe condition of disease.[42] In contrast, a cohort study by Rao et al.[43] demonstrated association between hypervirulent strain ribotype 027 with severe CDI disease (OR: 1.73, 95% CI 1.03 - 2.89; p = 0.037) and higher mortality rate (OR: 2.02, 95% CI 1.19 - 3.43; p = 0.009) juxtaposed to other ribotypes.[43] Study by See et al.[44] demonstrated similar results by using NAP1 strain, where analysis of multivariate regression depicted increased severity of CDI (OR: 1.66, 95% CI: 1.90 - 2.54) and higher mortality (OR: 2.12, 95% CI: 1.22 - 3.68).[44] Furthermore, a study in Quebec showed the hypervirulent strain ribotype 027 is associated with disease severity, twice more severe frequently in contrast to other strains. Nevertheless, basic reasons of these contradictory results were un-measured confounding factors, setting of study, detection methods of *C. difficile*, size of sample, population of study, and design of study. Therefore, the generalization of the study results has to be examined profoundly. Therefore, given these contrary findings, healthcare workers or providers advised to center their attention on infection treatment based on clinical reasoning and infection marker related to severe infection, as well as episodes of diarrhea, dehydration signs, albumin level, creatinine level, white blood cell (WBC) count, underlying comorbidities, and immunocompromised condition.[45,46]

## MECHANISM OF ENDEMIC STRAIN DISPLACEMENT WITH HYPERVIRULENT RIBOTYPE 027 *C. difficile* STRAIN

Transmission of pathogen occur via fecal-oral route with new infections emerge by bacterial spore consumption. *C. difficile* spores are resistance to desiccation and able to persist for about 5 months on hard or solid surface. Merrigan et al.[45] examined spore accumulation in regard to growth cycle of bacteria with results demonstrating that hypervirulent strains have the ability to sporulate faster and causing significant more spore accumulation per total volume compared to non-hypervirulent strains.[45] Increase sporulation rate could elucidate the uncommon soaring recurrence correlated to hypervirulent strains, 4-fold according to Marsh et al.[20,46], as patients tend to transmit the contamination to local surroundings, then re-infect themselves subsequently. Subsequently after dormant bacterial consumption and ingestion, germination of *C. difficile* spore occurs as exposure to combination of bile salts and L-glycine. Vegetative phase of *C. difficile* happen as colonization of host's gastrointestinal tract. Even tough colonization is required to cause the disease, most of infected people prevail asymptomatic. CDI manifestations are arbitrated by production of cytotoxic toxins to large intestine epithelial tissue lead the way of immense colon inflammation and epithelial cell obstruction of the host.[46] Study by Pepin *et al*.[47] and Hubert *et al*.[48] demonstrated doubling rate of severe disease as emergence of ribotype 027 in Canada. Hypervirulent strains associated

with higher rates of symptomatic disease presumed to be result of increased production of toxin or due to intensified variant clostridial toxins.[48] There are three probable mechanisms postulated in accordance to transitions from endemic strains to hypervirulent strains: (1) the more infectious strains are hypervirulent; (2) symptomatic condition with higher rate is caused by hypervirulent strain; (3) outcompete in host's gut can be done by hypervirulent strain.[49]

Throughout stochastic simulation, *C. difficile* hypervirulent strain invasion to human population cherished endemic strain was investigated. Reasoning of some previous models aims to establish infection control strategy in hospital and surroundings. Nevertheless, *C. difficile* has been recognized prominently as a global community pathogen, in preference to just segment of healthcare associated pathogen. In addition, present study has demonstrated major source of infection is community. Nonetheless, in some conditions if community not the primary source, infections suffered by high-risk group in healthcare environment. Underlying cause of difference between endemic strains and hypervirulent strains prevail undetermined regardless of current atypical strains constitute predominant infections in community surroundings. Therefore, the consequence of three distinct pathways of intensified were examined namely increasing pathogen infectiousness, increasing rate of colonization to symptomatic disease, and ability of endemic strains displacement by hypervirulent strains in colonized gut.[49,52] Instinctively, parameters govern these distinct mechanisms have positive correlation to possibility of establishment invading strains in community. Nevertheless, comparison of these parameters' influence on invasion rate and prevalence of resultant equipoise yielded different patterns of epidemiology. In accordance to classic epidemiological comprehension, the rate in which an establish pathogen spreading within susceptible individuals is strongly dependent on coefficient of transmission, as modelled by increasing the hypervirulent strain infectiousness. Simulation demonstrated increased infectious strains tend to establish further, spread robustly, and reach equilibrium to increased prevalence in community. Probability of successful established invasion and current steady circumstances of prevalence has been less influenced by rising colonized percentage on clinical disease experience. If individuals colonized by endemic strains were prone to hypervirulent strains, a weaker correlation was constructed with probability of establishment, and no comprehensible correlation was discerned with equilibrium prevalence outcome. Spreading of novel strain is substantially independent to endemicity of resident strain when gut is colonized by resident strain as uncolonized gut readily.[49-53]

Clinical reports over the past 15 years have demonstrated substantial increase of disease rate in accordance to prominent and robust switch in dominance of *C. difficile* strain. Isolates from PCR-ribotyping in Montreal hospital depicted NAP1/ribotype 027 were not found in 2000 and 2001. Nonetheless, NAP1/ribotype 027 constituted more than 75% isolates collected during the outbreak in 2003 until 2004. Increasing prevalence of CDI disease has corresponded to ribotype 027 dominance in many countries across the world, comprising England with its peak in 2007-2008, European countries, and North America.[49,50] Tying to epidemiological model with present analysis results, apparently hypervirulent strains' ability in displacing endemic strains from readily colonized host's gut is the slightest mechanism facilitates ribotype 027 dominance, resulting in more severe diarrhea and longer recovery period. In spite of investigating a wide range of parameter values, from resistance of colonization to susceptibility counterpart in uncolonized individuals, novel strain is unsuccessful to reproduce heightened level of prevalence associated with emerging hypervirulent strains. It does not invalidate the probability of more competitive hypervirulent strains compared to typical strains within host. However, it still suggests this mechanism is not a pivotal role for successful invasion and hypervirulent strain of ribotype 027 clonal dominance. Importantly, the present study depicted strains' competition inside host's gut is not essential for abrupt prevailing strains switching; all surrogate mechanisms

of hypervirulent distinctly illustrated previous dominant strains are not merely added on invasion of subsequent new strain, yet excluded throughout exploitative competition.[53,54]

## ANTIBIOTIC RESISTANCE OF HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN

Investigation of BI/NAP1/027 CDI cases in Panama showed high resistance to several antibiotics: rifampin, ciprofloxacin, levofloxacin, moxifloxacin, and clindamycin, yet remain susceptible to vancomycin and metronidazole. Study tested for several antibiotic susceptibility for ribotype 027 and non-027 ribotype in Canada with findings 92.2% resistance of ribotype 027 to moxifloxacin as opposed to 11.2% of other strains. Correspondingly, ribotype 027 strains (78.2%) showed resistance to ceftriaxone compared to other strains (15.7%). Ribotype 027 was greater than 4-fold higher of minimum inhibitory concentration (MIC) compared to metronidazole (4 μg/ml vs 1 μg/ml). In addition, ribotype 027 strain demonstrated 2-fold higher MIC of fidaxomicin (1 μg/ml vs 2 μg/ml). Nevertheless, resistance for vancomycin and clindamycin was akin both in group of BI/NAP1/027 and other strains. Erythromycin resistance is associated with mutation of methylase genes in ribosome, meanwhile fluoroquinolones resistance is caused by mutation of DNA gyrase. Resistance to fidaxomicin and rifamycin group is linked to methylation of ribonucleic acid (RNA) polymerase. In addition, resistance to linezolid is caused by genes of lincosamide and phenicol. Study in several hospitals in Mexico demonstrated numerous ribotype 027 isolates possesses decreased susceptibility to fidaxomicin even though this antibiotic is unavailable in Mexico and patients had been unexposed to it. Basis for BI/NAP1/027 strain treatment is antibiotics. Presently, specific guidelines of the Infectious Diseases Society of America (IDSA) remain unavailable to BI/NAP1/027 strain.[55-58] Consequently, based on overall CDI treatment guidelines, infection by BI/NAP1/027 strain treatment has been proposed as in Table 2.

**Table 2**. Suggestive antibiotic treatment for BI/NAP1/027 strain[55]

| | 1st line treatment | Alternative treatment |
|---|---|---|
| Initial non-severe infection | Vancomycin per oral (p.o.), 125 mg, 4 times daily, 10 days | Fidaxomicin p.o., 200 mg, twice per day, 10 days. If unavailable, take metronidazole, 500 mg, three times per day, 10 days |
| 1st non-severe recurrency | Vancomycin p.o., 125 mg, 4 times per day, 10 days | Oral fidaxomicin, 200 mg, twice per day, 10 days |
| 2nd non-severe recurrency | Vancomycin p.o. tapering off: 125 mg, 4 times, 7 until 10 days; 125 mg twice per day, 7 days; 125 mg once daily, 7 days; 125 mg per three days, 14 days | Fidaxomicin p.o., 200 mg, twice per day, 10 days, or transplantation of fecal microbiota |
| Later non-severe relapse | Transplantation of fecal microbiota | Vancomycin p.o. tapering off with probiotics, fidaxomicin, intravenous immune globulin (IVIG) |
| Severe disease | Vancomycin p.o. 125 mg, f4 times daily; rise to 500 mg, 4 times per day. This can be applied only if there is no improvement within 24 - 48 hours, or associated side effects, e.g., ileus, renal failure | If patient cannot tolerate vancomycin p.o, fidaxomicin is antibiotic of choice |
| Ileus | Plus intravenous metronidazole 500 mg, every 8 hours to fidaxomicin or oral vancomycin, consultation to general surgery should be considered | IVIG, intra colonic vancomycin |

## HYPERVIRULENT BI/NAP1/027 *C. difficile* STRAIN PREVENTATIVE MEASURES AND CONTROL

BI/NAP1//027 is well-known to cause outbreaks in hospital, and some reports have represented efforts and measures in regard to outbreak control. Muto[56] depicted combined measures to control outbreak in the University of Pittsburgh as a bundle of encompassed education; increment of early detection in regard to CDI requires nurses to make an order of toxin texting, and email notification to alert physicians who treat high risk patients, and establish a management team to evaluate patients tested positive for all *C. difficile* toxins. Expansion of infection control action comprises environmental cleansing with bleach, replacing alcohol hand rubs with water and soap to improve hand hygiene for CDI patients, continuation of contact precautions following diarrhea resolution, audit of infection control, and restriction of targeted antimicrobials.[56] Even though particular effect of each measures was difficult to ascertain, investigators delineated a 78% decrease of CDI incidence and severe CDI proportion. Furthermore, only 13% of *C. difficile* isolates were BI/NAP1/027 strain by 2005, compared to 51% among clinical isolates in 2001.[57-60]

In regard to numerous hospitals outbreak in Quebec, the Canadian government allocated $20 million to upgrade infection control measures in 12 hospitals; whereas in Pittsburgh, various approaches were implemented, comprising domestic measures intensification with thorough environmental cleaning of affected areas and toilets by applying bleach, cleaning all rooms on a subdivision or section if number of nosocomial occurrence exceeded within three weeks; equipment dedication; applying hand washing rather than alcohol rubs; prompt finding of CDI case by daily enhancement of toxin testing frequency in clinical laboratory; prompt empirical treatment and contact precaution practice subsequent to second diarrhea stool; move patients from 4-bed ward if possible; and education to decrease administration of cephalosporin and fluoroquinolone. Consequently, incidence of CDI in these hospitals declined from 22.5 to 12.4 per 1000 admittance as a result of applying these preventative measures, but incidence rates did not reach pre-outbreak extent.[61-65] One hospital in Quebec implemented antimicrobial stewardship when no effectivity was shown in decrease of CDI incidence after executing infection control measures. This unrestrained strategy leaned on education and commentary or assessment from pharmaceutical parties and hence attained administration reduction to 54% of total antibiotic and 23% of targeted antibiotic. Simultaneously, with diminishment in antibiotic consumption, CDI incidence has seen a 60% drop. Targeted antibiotics encompassed second and third generation of cephalosporin, macrolides, clindamycin, and ciprofloxacin. Drop in ciprofloxacin usage has been accompanied by increase. In other places, administration of moxifloxacinwas used as an agent incriminated as high-risk antimicrobial agent.[64,65]

Some factors contribute a significant role of therapy by fluoroquinolones in epidemics era, encompassing enhance resistance of BI/NAP1/027 strains to group of fluoroquinolones, juxtaposed to historical isolates not associated to epidemic isolates, expanded consumption of fluoroquinolones, along with high ascribable risk in regard to fluoroquinolones of this outbreak. However, considering assorted outcomes of certain fluoroquinolones restriction, un-assessed hypothesis that could be a "class effect," subsequently all fluoroquinolones restriction will be a specific potential control course of action in hospital with outbreak caused by BI/NAP1/027 strain. Various measures have been implemented in outbreak control of CDI, especially BI/NAP1/027 which poses a remarkable challenge.[66-68] Coalescence of elderly patients, continual use of antibiotics, contamination of hospital environment with spores are all ideal circumstances of CDI outbreaks, high rate or number of morbidity and mortality. Even though infection control measures, such as environmental cleaning, isolation, and hand hygiene, will persist as keystones course of action to prevent *C. difficile* exposure in hospital, methods to reduce disease

risk following *C. difficile* infection or ingestion have to be reckoned. Nevertheless, decreasing antibiotics use remains absolutely an important approach to reduce CDI risk. These measures have commenced in various hospitals, yet there is still considerable extent to improve antimicrobial stewardship. Methods to neutralize antibiotic disruption of microbiota colonization should be incorporated with biotherapeutic methods, e.g., nontoxigenic *C. difficile* strain administration which has demonstrated to be effective in hamster model. Focus measures also should emphasize on development of vaccine of *C. difficile* to boost immune state of elderly people. Passive immune methods such as monoclonal antibody to enhance immune response to toxin A and B seem to be effective in early stage of clinical trials. Nevertheless, even though current focus is on BI/NAP1/027 *C. difficile* strain, new upcoming epidemic strains are going to emerge in the foreseeable future.[69-72]

## CONCLUSIONS

Greater fatality and severity epidemics of CDI have been caused by highly virulent form of *C. difficile* of BI/NAP1/027 that spread widely and robustly over decades. Main risk factors are elderly age, hospitalization, and exposure to antibiotics, e.g., cephalosporins, fluoroquinolones, clindamycin, and penicillin. Virulence factors are TcdA, TcdB, CDTa CDTb; hypervirulence is prone to hypersporulation and mutation of TcD gene leads to toxin hyperexpression. Preventative measures can be done by early cases detection, building a management team to evaluate patient positive with all *C. difficile* toxins, hand hygiene improvement, continuation of contact precautions after diarrhea resolution, audit of infection control, restriction of antimicrobials, and development of a vaccine of *C. difficile*.

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## CONFLICT OF INTEREST

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***Original Article***

# An Overview of COVID-19 Patients in RSUD Bhakti Dharma Husada Surabaya from September 2020 to June 2021

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***ABSTRACT***

*The COVID-19 pandemic has been lasting more than a year. Until now, research on the analysis of an overview of COVID-19patients has not been carried out at RSUD Bhakti Dharma Husada Surabaya. This study aims to describe the COVID-19 cases in RSUD Bhakti Dharma Husada Surabaya about the gender of patients, highest number of patients =, the most recovered patient, the highest death rate occurred, and Case Fatality rate (CFR). This study is a descriptive observational study with a case series approach. The data used in this study were COVID-19 data from the application of online Hospital ditjen yankes from September 2020 to June 2021. The majority of COVID-19 cases occured in women (53.04 %). The COVID-19 patients mostly came to the hospital in June 2021, about 241. The most recovered patients in Oktober were 255 patients. The highest death rates occurred in June 2021 ware 47 patients. Case Fatality rate (CFR) is at 5.79 % because in June 2021 the health facilities were full, and cause patients did not get help quickly. Many patients have been forced to self-isolate at home so that they have worsened and finally died. Most COVID-19 patients who were treated at the RSUD Bhakti Dharma Husada Surabaya from 2020 to June 2021 occurred in women and the most patients who were admitted was in June 2021.*

***Keywords:*** *Descriptive, overview, patient, COVID-19, hospital*

***ABSTRAK***

*Pandemi COVID-19 telah berlangsung lebih dari satu tahun. Penelitian tentang analisis gambaran pasien COVID-19 di RSUD Bhakti Dharma Husada Surabaya hingga saat ini belum dilakukan. Penelitian ini bertujuan menggambarkan kasus COVID-19 yang ada di RSU Bhakti Dharma Husada Surabaya tentang jenis kelamin pasien, pasien yang paling banyak masuk rumah sakit, pasien yang paling banyak sembuh, angka kematian pasien paling tinggi dan Case Fatality rate (CFR). Penelitian ini merupakan penelitian deskriptif observasional dengan pendekatan case series. Sumber data pada penelitian ini adalah data OVID-19 terjadi pada perempuan (53.04%). Pasien COVID-19 paling banyak masuk pada bulan Juni 2021 sejumlah 241. Pasien paling banyak sembuh ada di bulan Oktober yaitu 255 pasien Terjadi angka kematian paling tinggi di bulan Juni 2021 sebanyak 47 pasien. Case Fatality rate (CFR) berada di angka 5.79 % sebab di bulan Juni 2021 fasilitas kesehatan penuh, sehingga pasien tidak segera mendapatkan perawatan. Banyak pasien yang terpaksa isolasi mandiri di rumah sehingga kondisinya semakin parah dan akhirnya meninggal dunia. PasienOVID-19 yang dirawat di RSUD Bhakti Dharma Husada Surabaya pada bulan September 2020-Juni 2021 paling banyak terjadi pada perempuan dan pasien paling banyak masuk pada bulan Juni 2021.*

***Kata kunci:*** *Deskrptif, gambaran, pasien,COVID d-19, rumah sakit*

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## INTRODUCTION

COVID-19 is a communicable disease firstly reported as Novel Coronavirus is as a caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).[1] Since declared a pandemic by WHO starting on March 11, 2020, until now the COVID-19 pandemic is still ongoing.[2] After Indonesia reported the first case on March 2, 2020, COVID-19 cases in Indonesia at the end of December 2020 had reached 743,198 people. 611,097 patients were recovered and 22,138 patients died. Cases are increasing and spreading rapidly throughout Indonesia, including Surabaya. As of July 15, 2021, the Surabaya City Covid-19 Task Force reported 32,297 confirmed COVID-19 cases with 1,433 deaths.[3,6]

RSUD Bhakti Dharma Husada is one of the COVID-19 referral hospitals with a capacity of 164 beds for COVID-19 patients. SIRS.kemkes.go.id first version has been used to collect data on COVID-19 patients in hospitals from March 2020 to August 2020. In the first version, the data collection is name, email, phone number, address, gender, age, date admission, patient status, date of discharge, discharge status, NIK, type of patient (Suspect, Confirmation), diagnosis, and laboratory examination. Since September 2020, SIRS.kemkes.go.id the second version has been used where data collection is in the form of daily data for triage ER patients, daily data for patients admitted, daily data for patients treated with comorbidities, daily data for patients treated without comorbidities, and daily data for patients discharged.[4]

This study aims to provide an analysis of the description of COVIDid-19 patients at the Bhakti Dharma Husada Hospital Surabaya as an input in handling COVID-19 cases in the city of Surabaya especially the Bhakti Dharma Husada Hospital Surabaya.

## MATERIALS AND METHODS

### Materials

This research was an observational descriptive study with a case series approach. The source of data in this study is secondary data taken from the online hospital application of the Directorate General of Health and Health version 2 (two) where the data started from September 2020 until the data collection for this study ended in June 2021. This study describes the incidence of COVID-19 with a case approach, epidemiology by person, and time. The variables studied in this study were gender, admitted patients, recovered patients, and deceased patients at Bhakti Dharma Husada Hospital Surabaya. The case fatality rate (CFR) variable is the result of the division between the number of confirmed COVID-19 deaths in a certain period and the number of confirmed COVID-19 cases in that period multiplied by 100% (WHO criteria).

## RESULTS AND DISCUSSION

In August 2020, RSUD Bhakti Dharma Husada had treated 554 confirmed COVID-19 patients (Figure 1).

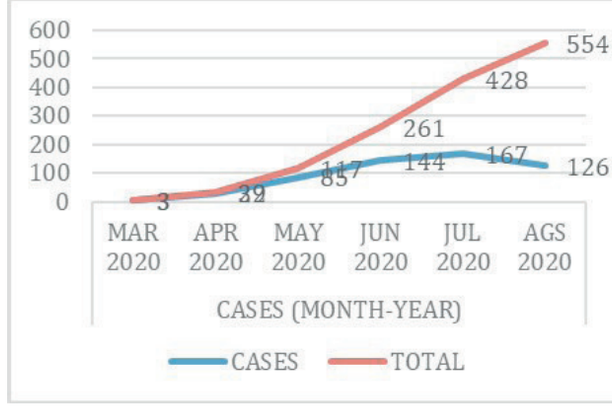


**Figure 1.** Coronavirus Cases March – August 2020

The numbers of COVID-19 patients who entered the Bhakti Dharma Husada Hospital in the period, March-August 2020 were the most in July namely 167 patients, and the lowest were in March with three patients.

The gender of COVID-19 patients who entered the Bhakti Dharma Husada Hospital in the period September 2020 - June 2021 the most were female, namely 436 patients (53.04%). Males gender was 386 patients (46.96%) as shown in Table 1.

The total numbers of COVID-19 patients who entered the Bhakti Dharma Husada Hospital in the period September 2020 - June 2021 were the most in June as many as 241 patients, and the lowest in March 2021 with 21 patients. The most recovered patients were in October about 255 patients, and the lowest in April 2021 with 15 patients. The highest death rate occurred in June 2021 namely 47 patients, the lowest in April and May 2021 namely 0 (zero) as shown in Fugre 2.

**Table 1.** Distribution of COVID-19 Cases Based on People at Bhakti Dharma Husada Hospital September 2020-June 2021

| CASES BY PEOPLE | CASES (MONTH-YEAR) | | | | | | | | | | AMOUNT | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SEPT 2020 | OCT 2020 | NOV 2020 | DEC 2020 | JAN 2021 | FEB 2021 | MAR 2021 | APR 2021 | MAY 2021 | JUN 2021 | | |
| **Gender** | | | | | | | | | | | n | % |
| Male | 44 | 27 | 19 | 64 | 45 | 25 | 7 | 9 | 12 | 133 | 386 | 46.96 |
| Female | 59 | 34 | 40 | 60 | 66 | 23 | 14 | 14 | 18 | 108 | 436 | 53.04 |
| Total | 103 | 62 | 59 | 124 | 111 | 48 | 21 | 23 | 30 | 241 | 822 | 100 |

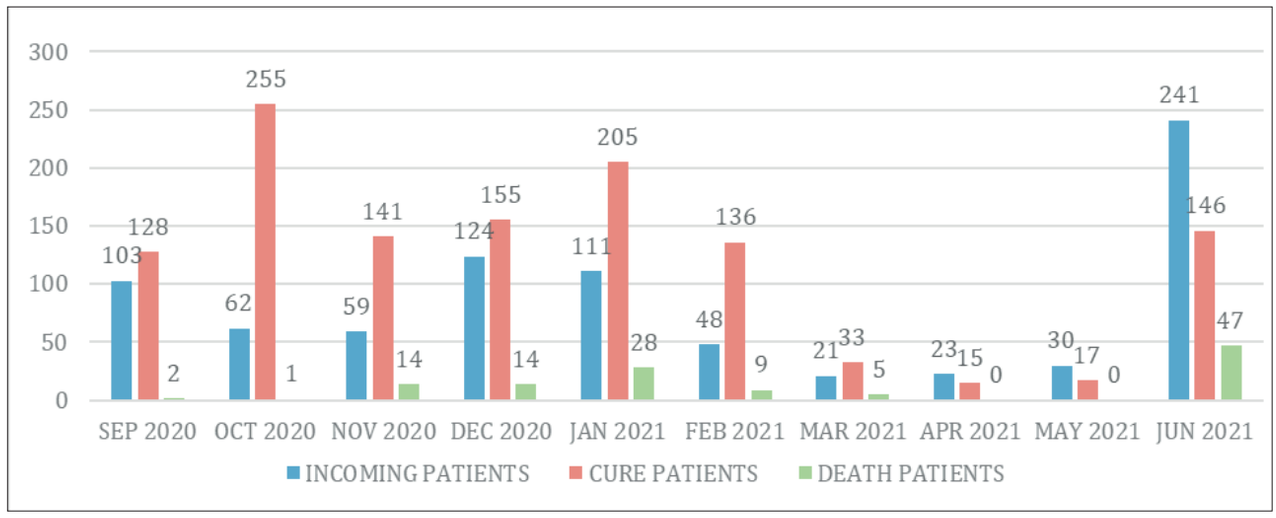


**Figure 2.** Distribution of COVID-19 Cases Based on Time at Bhakti Dharma Husada Hospital Surabaya September 2020-June 2021

**Table 2.** Distribution of Deaths of COVID-19 Patients at Bhakti Dharma Husada Hospital Surabaya September 2020-June 2021

| No | MONTH | CONFIRMED DIED PATIENT | CONFIRMED PATIENT | CFR(%) |
|---|---|---|---|---|
| 1 | SEPTEMBER 2020 | 2 | 197 | 1.02 |
| 2 | OCTOBER 2020 | 1 | 154 | 0.65 |
| 3 | NOVEMBER 2020 | 14 | 187 | 7.49 |
| 4 | DECEMBER 2020 | 14 | 344 | 4.07 |
| 5 | JANUARY 2021 | 28 | 350 | 8.00 |
| 6 | FEBRUARY 2021 | 9 | 162 | 5.56 |
| 7 | MARCH 2021 | 5 | 96 | 5.21 |
| 8 | APRIL 2021 | 0 | 80 | 0.00 |
| 9 | MAY 2021 | 0 | 57 | 0.00 |
| 10 | JUNE 2021 | 47 | 444 | 10.59 |
| AMOUNT | | 120 | 2017 | 5.79 |

According to the revised Ministry of Health COVID-19 guidelines, COVID-19 deaths for surveillance purposes are confirmed/probable COVID-19 cases that have died. The Case Fatality rate (CFR) of the Bhakti Dharma Husada Hospital in the period September 2020 - June 2021 was 5.79 % as shown in Table 2.

## COVID-19 case pattern based on gender

The gender of COVID-19 patients who entered the Bhakti Dharma Husada Hospital in the period September 2020 - June 2021 mostly were female, namely 436 patients (53.04%). It is also in line with WHO that the percentage of infection distribution in males is greater than in females (51% vs 47%) with some variations across age groups. Based on the data from 77, 000 deaths in the case-based reporting database (nearly 30% of all known deaths), there appear to be higher numbers of deaths (45,000 or 58%) in men. Geographical variations in infection rates and deaths among women and men of different age groups are probable; however, available data come from relatively few countries and are, therefore, skewed. Consequently, any interpretation of the gender differences across age groups and countries must be made with great caution. These limitations underline the urgent need for better and completed reporting of data by sex and age, as a minimum, for better identification and understand the key differences and disparities to inform a more effective COVID-19 response. Evidence from past epidemics, such as the SARS coronavirus outbreak in 2002−2003, shows that men and women are likely to have both different susceptibilities to the virus and different vulnerabilities to the infection as a result of both sex- and gender-related factors. Data (on persons tested, the severity of the disease, hospitalization rates, discharge [recovery], and health worker status) that are disaggregated at a minimum by sex and age – as well as by other stratifies such as socioeconomic status, ethnicity, sexual orientation, gender identity, refugee status, etc., where feasible – could help in identifying and addressing health inequities related to COVID-19.[27]

## COVID-19 case pattern based on time

According to WHO Science in 5 on COVID-19, some factors are contributing to increased transmission around the world. The first are these variants of concern, including the Delta variant which rapidly takes off and spreads between people more efficiently than even the Alpha variant that was first detected around December to January 2021. The second factor is that we have increased social mixing and increased

social mobility, which increases the number of contacts that individuals have. The third factor is the relaxation or the inappropriate use of public health and social measures. Proven public health and social measures we know prevent infections, reduce the spread of somebody who is infected with the virus to others, and save lives. And the fourth factor is the uneven and inequitable distribution of vaccines.[9]

### COVID-19 case fatality pattern

The results of the study show that more COVID-19 deaths occurred in June 2021 with 47 patients as shown in Table 2. One of the causes of the high number of cases of death is influenced by the increasing number of active cases in June 2021. This is because the health facilities were full, causing patients not to get help quickly. Many patients have been forced to self-isolate at home so that they have worsened and have been admitted to the hospital in severe conditions.

The results showed that, from September 2020-June 2021, the majority of COVIDd-19 cases occurred in June 2021, most patients recovered in October 2020 and most patients died in June 2021 as shown in Figure 2.

This study shows that the largest increase in cases and death rates of COVID-19 patients occurred in June 2021 where this occurred throughout Indonesia and the world.[3,5,6]

### COVID-19 case fatality pattern

According to Table 2, the total mortality of confirmed patients who died was 120 people (CFR 5.79%). Age, occupation (entrepreneur and farmer/trader), contact history, symptoms (fever, dyspnea, cough, lethargic, and cold), and comorbidities (diabetes, COPD, hypertension, cancer, heart disease, neurological disorders, and immune disorders) were risk factors of COVID-19 confirmed died patients in DR. Kariadi Hospital. Meanwhile, gender, traveling history, and duration of symptoms were not risk factors for death in COVID-19 confirmed patients in DR. Kariadi Hospital. Adequate handling is needed to prevent death in patients with confirmed COVID-19 who have risk factors. In another article,the mean case fatality rate for adults aged under 60 is estimated to be less than 0.2%, compared with 9.3% in those aged over 80. Even if comorbidities increased mortality risk by five times, the risk would remain lower for younger people than for most older adults.[11]

## CONCLUSIONS

The majority of COVID-19 patients treated at the Bhakti Dharma Husada Hospital from September 2020 to June 2021 were female ; 436 (53,04 %), The COVID-19 patients mostly came to the hospital in June 2021, about 241. The most recovered patients were in October namely 255 patients. The highest death rate occurred in June 2021 namely 47 patients. Case Fatality rate (CFR) is at 5.79 % because in June 2021 the health facilities were full, and cause patients did not get help quickly. Many patients have been forced to self-isolate at home so that they have worsened and finally died. the urgent need for better and completed reporting of data by sex and age, as a minimum, for better identification and understand the key differences and disparities to inform a more effective COVID-19 response. Assessment of the history of vaccine is very important. Based on what we know so far, vaccines are proving effective against existing variants, especially at preventing severe disease, hospitalization and death.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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**Research Article**

# Cat's Liver Disease Detection with SGOT and SGPT Evaluation as a Gold Standard Diagnosis

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***ABSTRACT***

*SGOT and SGPT are two enzymes found in the liver in large amounts. Therefore, elevated levels of these two enzymes in the blood indicate liver disease. This study aims to identify liver disease in cats in Surabaya through the levels of SGOT and SGPT in the blood as the gold standard of diagnosis. Samples came from stray cats and domesticated cats of random age, breed, and sex. The blood samples collected were 62 samples, consisting of 33 domestic cats and 29 samples from stray cats. This study showed that from 33 samples of domesticated cats, 19 samples had higher than normal levels of SGOT, and from 29 samples of stray cats, 27 samples had higher than normal levels of SGOT. For SGPT levels, from 33 samples of domesticated cats, six samples had higher than normal levels of SGPT, and from 29 samples of stray cats, six samples had higher levels of SGPT than average. Data analysis used an independent sample t-test with SPSS for Windows with a significance level of 0.05. The data analysis results showed no significant difference, which means that the high levels of cat SGOT and SGPT enzymes did not significantly affect the origin of the cat. Therefore, it can be concluded that high levels of SGOT and SGPT as the gold standard for detecting liver diseases can occur in all cats, including stray cats and domesticated cats.*

***Keywords:*** *SGOT, SGPT, blood chemistry, liver disease, cats*

***ABSTRAK***

*SGOT dan SGPT merupakan 2 enzim yang ditemukan pada organ liver dalam jumlah besar. Peningkatan kadar kedua enzim tersebut dalam darah, merupakan salah satu indikasi adanya penyakit pada liver. Penititian ini bertujuan untuk mengidentifikasi adanya penyakit liver pada kucing-kucing di Surabaya melalui kadar SGOT dan SGPT dalam darah sebagai gold standar diagnosis. Sampel berasal dari kucing liar dan kucing peliharaan dengan umur, breed, dan jenis kelamin acak. Koleksi sampel darah yang didapatkan sebanyak 62 sampel, terdiri dari 33 sampel berasal dari kucing peliharaan, dan 29 sampel berasal dari kucing liar. Hasil penelitian ini menunjukkan bahwa dari 33 sampel kucing peliharaan terdapat 19 sampel yang mempunyai kadar SGOT lebih tinggi dari normalnya dan dari 29 sampel kucing liar terdapat 27 sampel yang mempunyai kadar SGOT lebih tinggi dari normalnya. Untuk kadar SGPT, dari 33 sampel kucing peliharaan terdapat 6 sampel yang mempunyai kadar SGPT lebih tinggi dari normalnya dan dari 29 sampel kucing liar terdapat 6 sampel yang mempunyai kadar SGPT lebih tinggi dari normalnya. Analisis data menggunakan independent sample t-test dengan SPSS for Windows dengan taraf signifikasi 0,05. Hasil analisis data menunjukkan bahwa tidak terdapat perbedaan yang nyata yang artinya tingginya kadar enzim SGOT dan SGPT kucing tidak berpengaruh nyata terhadap asal usul kucing. Dapat disimpulkan bahwa kadar SGOT dan SGPT yang tinggi sebagai gold standar untuk mendeteksi penyakit liver dapat terjadi pada semua kucing, termasuk kucing liar dan kucing domestik.*

***Kata kunci:*** *SGOT, SGPT, kimia darah, penyakit liver, kucing*

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## INTRODUCTION

The liver is the largest organ in the body.[1] Two enzymes synthesized in the liver and found in large amounts are SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase). SGOT is an enzyme found in the cytosol of liver hepatocytes, but it is also present in the heart, skeletal muscle, kidneys and brain. Therefore, the examination of SGOT levels is a biochemical marker to determine the process of necrosis that occurs in the liver. SGPT is an enzyme whose direct synthesis is in liver tissue with the highest activity in the cytosol and mitochondria of hepatocytes. This enzyme is also found in skeletal muscle and cardiac cells, albeit in low concentrations.[2] Liver damage can cause SGOT and SGPT to leak into the bloodstream.[3] Thus, SGOT and SGPT can be better indicators than others to detect liver damage because these two enzymes will increase first, and the increase is more significant when compared to other enzymes.

In general, cats' lives are divided into two, that is, cats living by being kept by the community and cats living stray. Stray cats are cats whose breeding is not controlled, the population continues to increase, there are no owners, and they live roaming and foraging in public places that provide food.[4] Cats are included among crepuscular mammals that have been associated with humans for more than 9,500 years.[5] Like humans, the cat's body, is also composed of several systems to live everyday life, including the digestive system, musculoskeletal, nervous, endocrine, respiratory system, integumentary system, reproduction, secretory and urinary system, immune system, and circulatory system. Six organs work well to carry out the functions of the system type as well. One of the most important organs is the liver. The liver is a central organ because of its essential function, such as playing a role in regulating and regulating metabolism, hormone and protein synthesis, and influencing the immune response and clearing toxins from the bloodstream. A problem that affects liver function is liver disease.[7] The liver, with a similar role, also synthesizes the enzymes SGOT and SGPT. Thus, both stray cats and domesticated cats can be affected by liver disease. SGOT and SGPT are considered the most effective because these enzymes will increase first and more drastically when compared to other enzymes if the liver is damaged.[8]

A comparison is needed whether a cat that is properly cared for with a regular life, which we call a domesticated cat, will be at lower risk of liver disease than stray cats. So, this study aims to determine the levels of SGOT and SGPT obtained from cat blood samples from stray cats and domesticated cats in Surabaya through a blood chemistry laboratory examination. In addition, these results obtained evaluation materials to compare SGOT and SGPT values from the two, which are then used as a standard gold diagnosis for liver disease in cats.

## MATERIALS AND METHODS

### Materials

Cat blood samples randomly selected were obtained from two different environments, a total 33 samples from domesticated cats with pet owners living in Surabaya and 29 samples from stray cats from four different markets, namely: Pacar Keling Market, Pucang Market, Wonokromo Market and Keputran Market. A sampling of domesticated cat blood was carried out at the Physiological Laboratory of the Faculty of Veterinary Medicine, Wijaya Kusuma University, but for stray cat blood samples, it was directly carried out on the spot at the markets. Pacar Medical Laboratory Surabaya is a place to check the levels of SGOT and SGPT samples. All the research procedures were conducted from January to February 2021.

## Methods

### a. Sample Collection

The sample was used for a blood chemistry test to determine cat SGOT and SGPT levels in cat blood serum. Blood was taken as much as 1-3 cc using an IV catheter or a 3cc syringe at the location of the anterior antebrachial cephalic vein, saphenous vein, or jugular vein. The blood taken was accommodated in a plain tube (non-EDTA) so that serum was obtained.

### b. Laboratory Test

The tube containing the serum sample was then labelled and stored in a styrofoam box with icebox gel and then sent to the Pacar Medical Laboratory Surabaya for SGOT and SGPT examination.

### c. Data Analysis

The results of the data obtained were then tabulated, and compared with the reference values of normal cat SGOT and SGPT, so that data were obtained for cats that experienced an increase in SGOT and SGPT both in stray cats and in domesticated cats. Then it continued with the independent t-test with the SPSS program.[9]

## RESULTS AND DISCUSSION

The following are the levels of SGOT and SGPT from the results of laboratory examinations of domesticated cats and stray cats shown in Table 1.

**Table 1.** SGOT and SGPT Levels in Domesticated Cats

| NO | DATE | CAT'S NAME | SGOT (U/L) | DES | SGPT (U/L) | DES |
|---|---|---|---|---|---|---|
| 1 | 13/1/21 | K.YUKA | 141 | H | 422 | H |
| 2 | 13/1/21 | K.CIKI | 38 | H | 111 | |
| 3 | 13/1/21 | K.NINIS | 52 | H | 136 | H |
| 4 | 13/1/21 | K.UPRID | 32 | | 110 | |
| 5 | 20/1/21 | K.MILO | 42 | H | 89 | |
| 6 | 20/1/21 | K.IBI | 34 | H | 77 | |
| 7 | 20/1/21 | K.PESY | 21 | | 46 | |
| 8 | 20/1/21 | K.MILA | 179 | H | 477 | H |
| 9 | 20/1/21 | K.MEME | 22 | | 58 | |
| 10 | 20/1/21 | K.MOCHI | 24 | | 53 | |
| 11 | 20/1/21 | K.GEMBUL | 28 | | 124 | H |
| 12 | 20/1/21 | K.KEKE | 25 | | 175 | H |
| 13 | 20/1/21 | K.ONIX | 52 | H | 70 | |
| 14 | 20/1/21 | K.DUDUNG | 24 | | 25 | |
| 15 | 20/1/21 | K.KECIL | 47 | H | 39 | |
| 16 | 20/1/21 | K.KUMAL | 45 | H | 74 | |
| 17 | 20/1/21 | K.YELLO | 28 | | 56 | |
| 18 | 20/1/21 | K.HARUKA | 22 | | 50 | |
| 19 | 20/1/21 | K.COKI | 30 | | 67 | |
| 20 | 20/1/21 | K.MOMO | 25 | | 47 | |
| 21 | 20/1/21 | K.GENDUT | 33 | H | 63 | |
| 22 | 20/1/21 | K.DOSKI | 50 | | 64 | |
| 23 | 27/1/21 | K. SHAWN | 63 | H | 90 | |
| 24 | 27/1/21 | K. PIPO | 34 | H | 52 | |
| 25 | 27/1/21 | K. MIU | 71 | H | 69 | |
| 26 | 27/1/21 | K. KIMMI | 109 | H | 102 | |
| 27 | 27/1/21 | K. CICI | 32 | | 49 | |
| 28 | 27/1/21 | K. MOKI | 24 | | 52 | |
| 29 | 27/1/21 | K. SIKO | 40 | H | 59 | |
| 30 | 03/2/21 | K.MOEZA | 91 | H | 73 | |
| 31 | 03/2/21 | K. TONG | 185 | H | 390 | H |
| 32 | 03/2/21 | K.MARVEL | 57 | H | 75 | |
| 33 | 03/2/21 | K. MOI | 36 | H | 94 | |

* Normal lab values feline from Idexx lab
SGOT = 0.00-32.00 u/L (H= high)
SGPT=12-115 u/L (H= high)

**Table 2.** SGOT and SGPT Levels in Stray Cats

| NO | DATE | MARKET NAME | CODE | SGOT (U/L) | DES | SGPT (U/L) | DES |
|---|---|---|---|---|---|---|---|
| 1 | 21/1/21 | Pasar Pacar Keling Surabaya | PCK 1 | 65 | H | 97 | |
| 2 | 21/1/21 | | PCK 2 | 20 | | 40 | |
| 3 | 21/1/21 | | PCK 3 | 75 | H | 110 | H |
| 4 | 21/1/21 | | PCK 4 | 34 | H | 76 | |
| 5 | 21/1/21 | | PCK 5 | 38 | H | 57 | |
| 6 | 21/1/21 | | PCK 6 | 43 | H | 78 | |
| 7 | 21/1/21 | | PCK 7 | 80 | H | 123 | H |
| 8 | 21/1/21 | | PCK 8 | 45 | H | 68 | |
| 9 | 21/1/21 | | PCK 9 | 42 | H | 83 | |
| 10 | 21/1/21 | | PCK10 | 41 | H | 59 | |

| NO | DATE | MARKET NAME | CODE | SGOT (U/L) | DES | SGPT (U/L) | DES |
|---|---|---|---|---|---|---|---|
| 11 | 21/1/21 | Pasar Pucang Surabaya | PC 1 | 70 | H | 85 | |
| 12 | 21/1/21 | | PC 2 | 75 | H | 145 | H |
| 13 | 21/1/21 | | PC 3 | 32 | | 48 | |
| 14 | 21/1/21 | | PC 4 | 38 | H | 76 | |
| 15 | 21/1/21 | | PC 5 | 128 | H | 238 | H |
| 16 | 21/1/21 | | PC 6 | 45 | H | 60 | |
| 17 | 21/1/21 | | PC 7 | 58 | H | 67 | |
| 18 | 21/1/21 | | PC 9 | 65 | H | 149 | H |
| 19 | 21/1/21 | | PC 10 | 35 | H | 67 | |
| 20 | 04/2/21 | Pasar Keputran Surabaya | K1 | 48 | H | 64 | |
| 21 | 04/2/21 | | K3 | 60 | H | 59 | |
| 22 | 04/2/21 | | K8 | 86 | H | 116 | H |
| 23 | 04/2/21 | | K9 | 65 | H | 47 | |
| 24 | 04/2/21 | | K10 | 60 | H | 66 | |
| 25 | 04/2/21 | Pasar Wonokromo Surabaya | W1 | 42 | H | 66 | |
| 26 | 04/2/21 | | W2 | 78 | H | 79 | |
| 27 | 04/2/21 | | W5 | 50 | H | 55 | |
| 28 | 04/2/21 | | W7 | 64 | H | 89 | |
| 29 | 04/2/21 | | W8 | 68 | H | 93 | |

* Normal lab values feline from Idexx lab
SGOT = 0.00-32.00 u/L (H= high)
SGPT= 12-115 u/L

SGOT, SGPT, arginase, lactate dehydrogenase and Gamma Glutamyl Transaminase are enzymes present in the liver. Still, they are free to leave the cells and enter the blood vessels beyond their normal levels when damage to the liver parenchyma cell occurs as shown in Table 2. However, SGOT and SGPT are considered the most effective because these enzymes will increase first and more drastically when compared to other enzymes if the liver is damaged.[8] Examining the levels of SGOT and SGPT in domesticated and stray cats will be one indicator to detect the presence of liver disease in these cats.

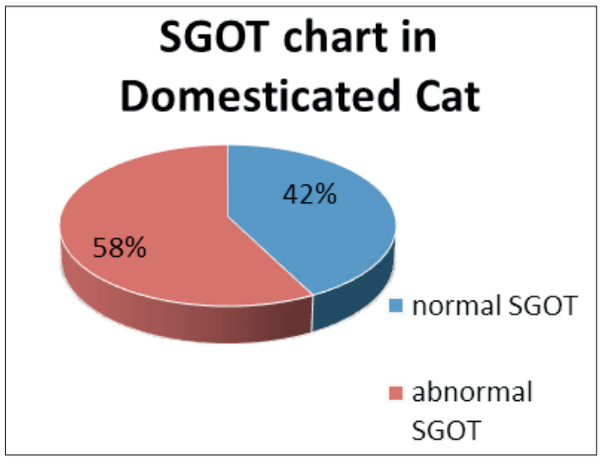


**Figure 1.** SGOT Chart in Domesticated Cat

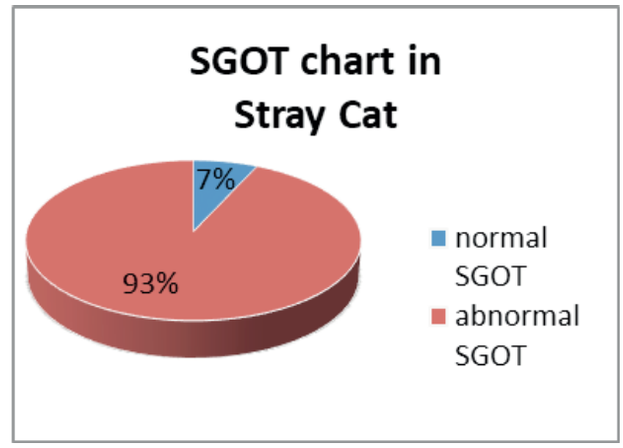


**Figure 2.** SGOT Chart in Stray Cat

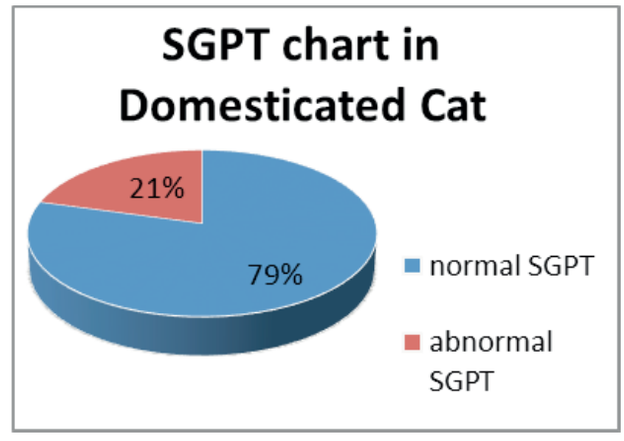


**Figure 3.** SGPT Chart in Domesticated Cat

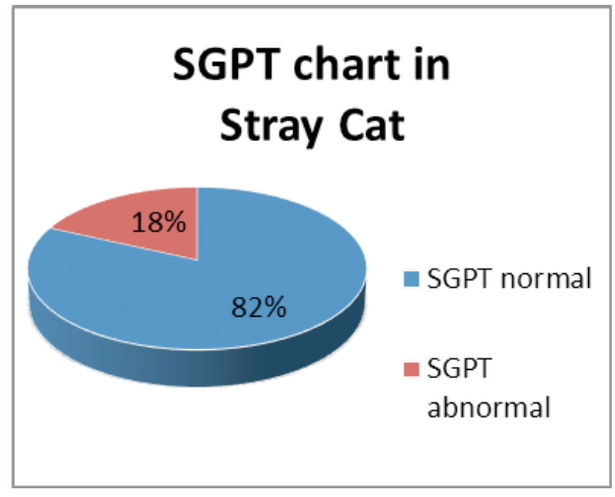


**Figure 4.** SGPT Chart in Stray Cat

By comparing the expected values of SGOT/AST and SGPT/ALT for cats the results of this study showed that, from 33 samples of

domesticated cats, 19 had higher levels of SGOT than expected (58%) as shown in Figure 1, and from 29 samples of stray cats, 27 had high levels of SGOT. In addition, SGOT was higher than expected (93%) as shown in Figure 2. For SGPT levels, from 33 domesticated cats, six samples had higher than normal levels of SGPT (21%) as shown in Figure 3, and from 29 samples of stray cats, six s had higher than normal levels of SGPT (18%) as shown in Figure 4.

**Table.3.** Mean Value SGOT in Domesticated Cat and Stray Cat

| Cat | Mean | Significance |
|---|---|---|
| **Domesticated** | 107.212 | T hit < t table<br>1.049 < 2.000<br>Sig. (2 tailed)<br>0.299 > 0.05<br>Not significantly |
| **Stray** | 84.827 | |

**Table. 4.** Mean Value SGPT in Domesticated Cat and Stray Cat

| Cat | Mean | Significance |
|---|---|---|
| **Domesticated** | 52.606 | T hit < t table<br>0,49 < 2,000<br>Sig. (2 tailed)<br>0,626 > 0,05<br>Not significantly |
| **Stray** | 56.896 | |

Analysis of independent t-test data SGOT value of all samples Sig. 2-tailed > 0.05 with a result of 0.626 > 0.05, indicating that the SGOT value in domesticated and stray cats was not significant as shown in Table 3. This also applies to the SGPT value with 0.299> 0.05, which means no significant difference between the SGPT value in domesticated and stray cats as shown in Table 4. From these results, it is explained that both stray cats and domestic cats can experience liver problems. This is evident from the effects of increased levels of SGOT and followed by levels of SGPT, which significantly increased above the average experienced by some cats. The most drastic increase in domesticated cats occurred in Yuka's cat, with an SGOT level of 141 u/L and an SGPT level of 422 u/L. Meanwhile, the most drastic increase in stray cats was cat blood samples taken from the Pucang market with an SGOT level of 128 u/L and an SGPT level of 238 u/L.

Evaluation of the SGOT and SGPT examinations results is one of the essential indicators for diagnosing liver disease in cats, just like humans. Because when the liver is damaged, the enzymes SGOT, SGPT, arginase, lactate dehydrogenase and Gamma Glutamyl Transaminase are free to leave the cells to enter the blood vessels more than expected and their levels in the blood increase.[10,11] Although there are other enzymes, SGOT and SGPT will increase first, and the growth is more extreme when compared to other enzymes.[8] The markers of liver cell abnormalities (hepatocellular) are caused by changes in permeability or damage to liver cell walls, increasing SGPT or SGOT.[12] Increased SGOT can persist in the circulation between 2-5 days, and so it is used as a biochemical marker to determine the presence of necrosis in liver cells.[2] Although SGOT and SGPT examinations from blood results are the gold standards to indicate liver disease, other supporting diagnoses are also needed, such as x-ray results, ultrasound, results of bilirubin, and Gamma Glutamyl Transaminase enzymes level, etc.

Liver disease in cats has several factors that can cause SGOT and SGPT enzymes to increase, for example, viral liver disease, liver ischemia caused by prolonged hypotension or acute heart failure, and heart damage due to drugs or toxins.[13,14] For example, the toxic effects of paracetamol in cats, which can come from a single-dose or cumulative dose manifested in methemoglobinemia and liver problem.[15] Paracetamol is metabolized in the liver, and the rest is metabolized in the kidney. [16] Cats with an amount of 10 mg/kg B.W. can cause symptoms of paracetamol poisoning. That matters because the cat cannot metabolize paracetamol due to the deficiency of the enzyme glucuronyl transferase.[17] Paracetamol contains NAPQI compounds that cannot be detoxified so that they form free radical toxic proteins and cause damage to cat liver cells.[18] In addition, paracetamol overdose can also cause hepatic cell necrosis in the centrilobular area, which causes acute liver failure.[19] An example of an organophosphate that farmers often use is diazinon.[20] Diazinon toxin will cause various damage in tissues, especially

in organs that function as detoxification, namely the liver.[21]

Another factor that can lead SGOT and SGPT enzymes to increase and cause liver damage is the entry of pathogenic microorganisms such as bacteria, viruses, fungi and parasites. A fungal microorganism that causes candidiasis, Candida albicans causes cases of hepatitis in male domesticated cats. As a result of this infection, the cat develops progressive hyperbilirubinemia, a nearly 10-fold increase in SGOT/AST and an SGPT/ALT increase of more than 18-fold from ordinary.[22]

Changes in SGOT and SGPT levels are related to the rate of protein metabolism; the level of physical activity may also influence cell generation.[23] Protein metabolism is related to the liver because it can adjust protein production with the body's protein requirements. The protein content in the blood is influenced by age and growth, nutrition intake, gender, hormones, pregnancy and lactation, stress, and fluid loss.[24] Likewise, the age factor affects total protein levels; at a young age, total protein levels tend to be higher.[25]

Strenuous physical activity can damage more muscle cells than the intermediate physical state acting correctly; it causes the occurrence of excessive circulation of SGOT in blood, because large amounts of serum glutamate oxaloacetate transaminase (SGOT) are found in muscle cells, liver cells, and heart muscle. And small amounts are found in other cells, such as cells of the kidney, pancreas, brain and erythrocytes.[26] So, it is necessary to have other continuous parameters to determine the presence of liver necrosis, that is, to check the levels of SGPT which have been considered a sensitive marker of liver disease and hepatotoxicity compared to SGOT levels.[27] Other factors such as obesity, genetics, and immune system disorders can also cause liver disease, driving an increase in SGOT and SGPT in the blood.

## CONCLUSIONS

High SGOT and SGPT as the gold standard for detecting liver diseases can occur in all cats, including stray cats and domesticated cats, because these two enzymes increase to the most extreme and earlier than other enzymes when the liver is impaired. Other supporting diagnoses are also needed, such as x-ray results, ultrasound results, or results of bilirubin and Gamma Glutamyl Transaminase enzymes level.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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***Original Article***

# Characteristics of Chronic Sinusitis Based on Non-Contrast CT Scan at the ENT-Head and Neck Surgery Polyclinic of Regional General Hospital Dr. Zainoel Abidin Banda Aceh

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***ABSTRACT***

*Chronic sinusitis is a long-term inflammation that occurs in the nasal and paranasal mucosa for 12 weeks. Non-contrast CT scan is gold standard in diagnosing chronic sinusitis. This study aims to determine the characteristics of chronic sinusitis based on non-contrast CT scan at the ENT-Head and Neck Surgery Polyclinic of RSUDZA Banda Aceh in 2019. This research was a descriptive study with retrospective data, medical record. The sample of this study was taken by consecutive sampling method in October 2020 and obtained 111 samples. The results showed that most patients with chronic sinusitis were 30-39 years), as many as 42 people (37.8%). Most of the sexes suffering from chronic sinusitis were women, as many as 59 people (53.2%). Based on the non-contrast CT scan, the location of the sinuses most affected was the maxillary sinuses, as many as 110 people (99.1%). The number of sinuses that were most affected was single sinusitis, which was 58 people (52.3%). Most patients with chronic sinusitis without polyps were found, as many as 89 people (80.2%). The most common anatomical variation found was septal deviation as many as 25 people (22.5%). The conclusions in this study indicate that women, late adulthood, maxillary sinus, single sinusitis, chronic sinusitis without nasal polyps, and septal deviation are characteristics of chronic sinusitis patients based on non-contrast CT scan.*

***Keywords**: Chronic Sinusitis, Non-contrast CT Scan, RSUDZA, Aceh*

***ABSTRAK***

Sinusitis kronis merupakan inflamasi jangka panjang yang terjadi pada mukosa nasal dan paranasal selama 12 minggu. *Pemeriksaan penunjang gold standard dalam menegakkan diagnosis sinusitis kronis adalah CT Scan tanpa kontras. Penelitian ini bertujuan untuk mengetahui karakteristik penderita sinusitis kronis berdasarkan gambaran CT Scan tanpa kontras di Poliklinik THT-KL RSUDZA Banda Aceh pada tahun 2019. Jenis penelitian ini adalah penelitian deskriptif dengan rekam medis. Sampel diambil dengan teknik consecutive sampling dan didapatkan 111 sampel. Hasil penelitian ini mendapatkan bahwa penderita sinusitis kronis paling banyak dialami pada umur 30-39 tahun yaitu sebanyak 42 orang (37.8%). Jenis kelamin yang paling banyak menderita sinusitis kronis yaitu perempuan sebanyak 59 orang (53.2%). Berdasarkan gambaran CT Scan tanpa kontras, letak sinus yang paling banyak terkena yaitu sinus maksilaris sebanyak 110 orang (99.1%). Jumlah sinus yang paling banyak terkena yaitu single sinusitis sebanyak 58 orang (52.3%). Penderita sinusitis kronis tanpa polip nasi paling banyak ditemukan yaitu sebanyak 89 orang (80.2%). Variasi anatomi yang paling banyak ditemukan adalah deviasi septum nasi yaitu sebanyak 25 orang (22.5%). Kesimpulan pada penelitian ini menunjukkan bahwa perempuan, usia dewasa akhir, sinus maksilaris, single sinusitis, sinusitis kronis tanpa polip nasi, dan deviasi septum merupakan karakteristik dari penderita sinusitis kronis berdasarkan gambaran CT Scan tanpa kontras.*

**Kata kunci**: *Sinusitis kronis, CT Scan tanpa kontras, RSUDZA, Aceh*

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## INTRODUCTION

Sinusitis, better known as rhinosinusitis, is an inflammation that occurs in the paranasal sinuses.[1] The cause can be due to infection, allergies, or autoimmune problems. In some case studies, viral infection was the most common cause and resolved within 10 days.[2] Sinusitis was classified by duration as acute if less than four weeks and chronic if more than 12 weeks with or without acute exacerbations.[3]

Chronic sinusitis has two or more the following symptoms, such as nasal congestion, nasal discharge (anterior/posterior nasal drip), facial tenderness or facial pain, and a decreased sense of smell.[4] The most common risk factor is allergies. While others are asthma, pollution and smoke exposure, immune deficiency, and septal deviation.[5]

Sinusitis and chronic sinusitis are the most common public health problems worldwide.[4] On 107 million people who suffer from chronic sinusitis in mainland China in 2015 showed that chronic sinusitis is common among people with certain medical conditions, including allergic rhinitis, asthma, chronic obstructive pulmonary disease, and gout. The prevalence of men (8.79%) is higher than women (7.28%). The independent risk factors for chronic sinusitis were active smokers and passive smokers. Therefore, it is necessary to develop health promotion related to chronic sinusitis, especially in developing countries.[5]

According to the 2007 National Health Interview Survey data, sinusitis is one of the ten most diagnosed diseases in the United States.[6] In Europe, about 10.9% of people have symptoms of chronic sinusitis.[7] In Canada, 5% of the general population suffers from chronic sinusitis.[8]

In Indonesia, based on data from the Ministry of Health of the Republic of Indonesia in 2003, there were 102,817 sinus patients undergoing outpatient treatment, while nasal and sinus disease was ranked 25th out of 50 major disease patterns. A study by Amelia et al.[7] in 2017 showed 73 patients with chronic sinusitis for one year at Dr. Mohammad Hoesin Palembang.[7] The Aceh provincial health profile noted that sinusitis was ranked 11th out of the 20 most diseases for outpatients in Aceh Provincial hospitals in 2012 with 8,183 cases.[9] A study by Husni and Pradista[10] in 2012 at the Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia showed that there were 33 sufferers of chronic sinusitis from October to December 2010.[10]

In establishing the diagnosis of chronic sinusitis, an objective examination is necessary because the symptoms that appeared could be non-specific.[4] The essential examinations for sinusitis are anterior rhinoscopy, nasoendoscopy, and radiological imaging. Radiological imaging involved paranasal sinuses x-ray, paranasal sinuses Computed Tomography (CT) Scan, and Magnetic Resonance Imaging (MRI). The radiologic examination is often necessary to confirm chronic sinusitis.[11] However, the CT scan of the paranasal sinuses is the gold standard in confirming the diagnosis of chronic sinusitis.[12] Mucosal abnormalities, sinus ostium obstruction, anatomic variations, and nasal polyps can be depicted well by CT scan.[3] However, the disadvantages of CT scan are its relatively high cost and the large radiation dose.[13]

This study was conducted at the Regional General Hospital Dr. Zainoel Abidin (RSUDZA) Banda Aceh, a referral hospital in Aceh. There has never been such a similar study before. Based on the description above, we are interested to learn more about the characteristics of patients with chronic sinusitis based on CT scan images without contrast at the ENT-Head and Neck Surgery polyclinic, RSUD Dr. Zainoel Abidin, Banda Aceh, Indonesia.

## MATERIALS AND METHODS

This descriptive study was conducted using retrospective data from medical records, describing age, gender, location of the affected sinus, number of affected sinuses, presence of nasal polyps, and anatomical variations based on non-contrast CT scan. This study was located at the Regional General Hospital DR. Zainoel Abidin Banda Aceh, precisely at the ENT-Head and Neck Surgery Polyclinic and Radiology Installation. This study was held from May to December 2020, with data collection time from 23 September to 13 October, 2020.

The population in this study were adults with symptoms of chronic sinusitis. The patients were treated at the ENT-Head and Neck Surgery Polyclinic, RSUD DR. Zainoel Abidin Banda Aceh in 2019. The sample in this study was patients with chronic sinusitis who met the inclusion and exclusion criteria. The sampling method of this study was using a non-probability side method or the consecutive sampling method. Univariate analysis was used to obtain the frequency distribution and the percentage of the variables studied.

## RESULTS AND DISCUSSION

This study was conducted at the ENT-Head and Neck Surgery Polyclinic and the Radiology Installation of RSUD Dr. Zainoel Abidin Banda Aceh, in September and October 2020. The number of outpatients who had symptoms of chronic sinusitis and went to the ENT-Head and Neck Surgery Polyclinic RSUDZA in 2019 amounted to 146 people; however, there were 35 samples that could not be used because they did not meet the inclusion criteria, so that the total sample in this study amounted to 111 people with the following characteristics as shown in Table 1.

Based on Table 1, the majority of the respondents were aged 30-39 years among 42 people (37.8%). The results of this study are in accordance with the study conducted by Julyanti that most chronic sinusitis occurs at the age of 31-40 years in a sample of 30 people (25.2%).[17] A study by Sogebi[18] found that the age group of 31-45 years was often affected by chronic sinusitis using a sample of 48 people (33.6%).[18] Moreover, a study by Pirzadeh et al.[19] found chronic sinusitis mostly occurred in the age group of 30-39 years in a sample of 25 people (30.1%)[19] Adults are more involved in outdoor activities and more at risk of exposure to the allergens or pollution that may cause or exacerbate chronic sinusitis.[20]

**Table 1.** Characteristics of Patients with Chronic Sinusitis by Age

| Age (year) | n | % |
|---|---|---|
| 20-29 | 24 | 21.6 |
| 30-39 | 42 | 37.8 |
| 40-49 | 23 | 20.7 |
| 50-59 | 22 | 19.8 |
| Total | 111 | 100 |

**Table 2.** Characteristics of Chronic Sinusitis by Gender

| Gender | n | % |
|---|---|---|
| Male | 52 | 46.8 |
| Female | 59 | 53.2 |
| Total | 111 | 100 |

Based on Table 2, 59 female patients (53.2%) had chronic sinusitis in line with the study by Trihastuti et al.[21] of 38 women (60.32%), compared to 25 men (39.68%).[21] In a sample of 42 people, Aritonang[22] also found that more women (52.5%) suffer from chronic sinusitis.[22] A study by Pirzadeh et al.[19] of 49 subjects (women 55.4%, men 44.6%)[19] found women are more likely to have a high level of concern for health, thus, women visit health services more often.[14] Women were also more susceptible to infection and obstruction due to the small size of the sinus ostium.[23] However, Amelia et al.[7] found that of 73 people, chronic sinusitis was more commonly found in men (43, 58.9%) than women (30, 41.1%) with the ratio of male and female with chronic sinusitis 1.4:1.[7] Men have more smoking habit and are more often exposed to pollution than women.[23]

Based on the non-contrast CT scan, the characteristics of patients with chronic sinusitis showed the location of the affected sinus, the number of affected sinuses, the presence of nasal polyps, and anatomical variations. The characteristics of patients with chronic sinusitis based on a non-contrast CT scan shown in Table 3.

**Table 3.** Distribution of Affected Sinuses

| Location of the affected sinus | Yes | | No | |
|---|---|---|---|---|
| | n | % | n | % |
| Sinus frontalis | 32 | 28.8 | 79 | 71.,2 |
| Sinus ethmoidalis | 51 | 45.9 | 60 | 54.1 |
| Sinus maksilaris | 110 | 99.1 | 1 | 0.9 |
| Sinus sphenoidalis | 26 | 23.4 | 85 | 76.6 |

Based on Table 3, it was found that the location of the sinus that was most affected was the maxillary sinus in a sample of 110 people (99.1%). Fadda and Aversa explain that the maxillary sinus is the sinus that is most often involved in chronic sinusitis.[15] Kurniasih and Ratnawati[24] also found that the maxillary sinus was the most common sinus in a sample of 106 people (86.89%).[24] Enema Job also remarked that the maxillary sinus was the most frequently involved sinus in a sample of 49 people (81.7%), followed by the ethmoid sinus in f 41 people (68.3%), frontal sinus in f 24 people (40%), and the least was the sphenoid sinus in 12 people (20%).[25] The maxillary sinuses have an ostium that is located higher than the sinus base, thus the maxillary sinus drainage depends on the ciliary function. If an infection occurs, it will impair the ciliary function and interfere with sinus drainage which will eventually lead to chronic sinusitis.[26] The inferior wall of the maxillary sinus is also adjacent to the roots of the 1st and 2nd molars, which can cause minor elevations or spots. Protruding along the maxillary sinus. The anatomic relationship of the maxillary molars to the maxillary sinus may facilitate the development of periapical or periodontal odontogenic infection within the maxillary sinus. Maxillary sinus may facilitate the development of periapical or periodontal odontogenic infection within the maxillary sinus.[27]

**Table 4.** Distribution of the Number of Sinuses Affected

| Sinuses affected | n | % |
|---|---|---|
| *Single* sinusitis | 58 | 52.3 |
| Multisinusitis | 35 | 31.5 |
| Pansinusitis | 18 | 16.2 |
| Total | 111 | 100 |

Table 4 shows the number of sinuses affected was single sinusitis, which was 58 people (52.3%). Makusidi found that single sinusitis often occurred in 86 people (58.9%).[28] Nova Sitinjak also found 104 cases of single sinusitis (63.8%).[29] However, Multazar et al.[30] stated that chronic sinusitis was more common in multiple sinuses (multisinusitis) in a sample of 22 people (88%). This may be related to the osteomental complex (KOM) as the final route of drainage from the frontal sinus, maxillary sinus, and ethmoidal sinus. Thus, if there are some disturbances in KOM, such as inflammation or edema, this will allow the occurrence of chronic sinusitis in several sinuses (multisinusitis).[16,30]

**Table 5.** Distribution of Nasal Polyps

| Nasal Polyps | n | % |
|---|---|---|
| Chronic Sinusitis With nasal polyps | 22 | 19.8 |
| Chronic Sinusitis without nasal polyps | 89 | 80.2 |
| Total | 111 | 100 |

Based on Table 5, it was found that chronic sinusitis without nasal polyps was more common than chronic sinusitis with nasal polyps in a sample of 89 people (80.2%). This is in accordance with Benjamin et al who also found that 507 people (82%) had chronic sinusitis without nasal polyps while 111 people (18%) had chronic sinusitis with nasal polyps.[31] Cho et al.[32] also stated that chronic sinusitis without nasal polyps is more common than chronic sinusitis with nasal polyps.[32] However, Rowe[33] also stated that nasal polyps are only involved in 15-20% of patients.[33] Chronic sinusitis without nasal polyps is more common than chronic sinusitis with nasal polyps. Chronic sinusitis without nasal polyps is characterized by edema and

inflammation of the sinuses which can be caused by several factors, such as allergies, irritation, and infection, while chronic sinusitis with nasal polyps is characterized by a soft mass formed from the mucous membrane in the nasal cavity called nasal polyps. These polyps can become large enough to block the sinuses and cause sinusitis symptoms.[34] The inflammatory reaction in chronic sinusitis without nasal polyps is Th1 and Th2 mediated, whereas chronic sinusitis with nasal polyps is Th2 dominant, which is characterized by high tissue eosinophilia. It is generally accompanied by an increase in tissue mast cells, innate lymphoid cells, immunoglobulin E, and Th2 cytokines.[35] Chronic sinusitis without nasal polyps shows basal membrane thickening, goblet cell hyperplasia, subepithelial edema, and mononuclear cell infiltration. Meanwhile, chronic sinusitis with nasal polyps shows epithelial damage, edema, and a reduced number of blood vessels and glands.[31]

**Table 6.** Distribution of Anatomical Variations

| Anatomical Variation | Yes | | No | |
|---|---|---|---|---|
| | n | % | n | % |
| Septal Deviation | 25 | 22,5 | 86 | 77.5 |
| Konka bulosa | 3 | 2.7 | 108 | 97.3 |
| Konka media paradoks | 0 | 0 | 0 | 0 |
| Haller Cell | 0 | 0 | 0 | 0 |
| Agger Nasi Cell | 0 | 0 | 0 | 0 |
| Onodi Cell | 0 | 0 | 0 | 0 |

Table 6 shows that the most anatomical variation was the septal deviation among 25 people (22.5%). This is in accordance with Shivakumar et al, that the most common anatomical variation was the septal deviation among 98 people (71%).[36] Moreover, Ratnawati[37] stated that septal deviation was the most common anatomical variation among 24 people (77%).[37] Aramani et al.[38] also found that 40 people (74.1%) had a deviated septum and 18 people (33.3%) were chronic sinusitis patients.[39] Septal deviation is an anatomical variation that is often found and one of the predisposing factors for chronic sinusitis. The presence of nasal septal deviation increased airflow around the osteomental complex (KOM), which can result in disruption of the mucociliary clearance process.[5,40] Ajmal[41] found that C-shaped deviation was the type that caused the most chronic sinusitis of 62.5% of 150 patients with chronic sinusitis, while the S-shaped deviation can cause pansinusitis because the S-shaped deviation can block the flow of air in both noses.[41] While concha bullosa is pneumatization that occurs in the concha media and can narrow the semilunar hiatus and block the infundibular drainage, resulting in sinusitis.[42] The bullous conchae are small with a vertical height of less than 50% of the total median conchae as measured on a coronal CT scan. While the bullous conchae are said to be large if the vertical height is more than 50% with an increase in the volume of the media conchae. Do Santos[43] found large bullous turbinates were more than small bullous cones in a sample of 222 people (80%).[43]

The paradoxical middle turbinate is a condition when the median turbinate bends laterally and can lead to narrowing of the COM and chronic sinusitis.[38] Haller cells or infraorbital ethmoid cells are located between the maxillary and orbital sinuses. It can increase the risk of orbital injury during ethmoidectomy. Haller cells potentially narrow the maxillary sinus ostium or ethmoid infundibulum which can obstruct the ostium.[16] Agger nasi cells are the most anterior ethmoidal cells that can narrow the frontal recess and block the frontonasal duct causing sinusitis.[40] Onodi cells are the rarest anatomical variation and can extend to the sphenoid sinus and surround the optic nerve. It is necessary to be careful in performing functional endoscopic sinus surgery.[39]

## CONCLUSSIONS

Patients with chronic sinusitis mostly are aged 30-39 years and primarily women. Based on a CT scan without contrast, the most affected sinus is the maxillary sinus. The number of sinuses affected in chronic sinusitis is single sinusitis. Chronic sinusitis without nasal polyps is more common than chronic sinusitis with nasal polyps. The most anatomical variation is nasal septal

deviation. The number of patients with chronic sinusitis based on CT scan images without contrast in 2019 was 111 patients.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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***Original Article***

# Correlation Between Risk of Febrile Neutropenia Based on Rondinelli Score with Clinical Outcomes in Acute Lymphoblastic Leukemia Patients

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***ABSTRACT***

*Febrile neutropenia (FN) is the most severe complication in patients with blood cancer and chemotherapy. Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children and the most common cause of febrile neutropenia. The low number of neutrophils in ALL patients due to lymphoblast cancer cells and the toxicity of chemotherapy makes patients susceptible to infection which, if not treated immediately, can lead to death. Early risk assessment for infectious complications in FN patients is needed to increase clinician awareness in high-risk patients and eliminate unnecessary therapy for low-risk patients. The Rondinelli scoring system is a reasonably good instrument for predicting severe infectious complications in pediatric patients with ALL who have febrile neutropenia. This study aims to determine the relationship between the risk category for febrile neutropenia (FN) based on the Rondinelli score with clinical outcomes in FN patients with acute lymphoblastic leukemia (ALL) in the Hematology-Oncology division of the child health department of RSUD Dr. Soetomo. This analytic observational study used secondary data FN patients with acute lymphoblastic leukemia (ALL) implementing a total sampling. From 30 samples of pediatric ALL patients with febrile neutropenia at Dr. Soetomo Hospital for June 2018-June 2020 it was found 17 patients (56.7%) had a moderate risk score category, and 13 others were in a low-risk category (43.3 %). Patients were dominated by moderate and severe severity of neutropenia respectively, 43.3%, had neutropenia for 1-7 days (50%), fever less than seven days (66.7%), had a length of stay of 8-14 days, and 15-30 days 33.3% each. Conclusion from this research is that there was a significant relationship between the Rondinelli score category in pediatric ALL patients with FN with the severity of neutropenia p=0.037; R=0.383), duration of neutropenia (p=0.021; R=0.420), duration of fever (p=0.000; R=0.618), and length of stay (p-value 0.005; R=0.496).*

***Keywords:*** *Febrile neutropenia; acute leukemia lymphoblastic; cancer; Rondinelli score; infection*

***ABSTRAK***

*Demam neutropenia (DN) merupakan komplikasi terberat pada pasien kanker darah dan kemoterapi. Leukemia limfoblastik akut (LLA) adalah jenis kanker yang paling umum pada anak-anak dan penyebab tersering dari demam neutropenia. Rendahnya jumlah neutrofil pada pasien LLA yang diakibatkan sel kanker maupun toksisitas kemoterapi , mengakibatkan pasien mudah terinfeksi yang apabila tidak segera ditangani dapat menyebabkan kematian. Penilaian risiko dini untuk komplikasi infeksi pada pasien DN diperlukan untuk meningkatkan kewaspadaan klinisi pada pasien berisiko tinggi dan mengeliminasi terapi yang tidak perlu untuk pasien berisiko rendah.. Sistem penilaian Rondinelli adalah instrumen yang cukup baik untuk memprediksi komplikasi infeksi berat pada pasien anak dengan LLA yang mengalami demam neutropenia. Penelitian ini bertujuan untuk mengetahui hubungan antara kategori risiko demam neutropenia (DN) berdasarkan skor Rondinelli dengan luaran klinis pada pasien leukemia limfoblastik akut (LLA) di divisi Hematologi-Onkologi departemen kesehatan anak RSUD Dr. Soetomo. Penelitian observasional analitik ini menggunakan data sekunder pasien DN dengan leukemia limfoblastik akut (LLA) yang dilakukan secara total sampling. Dari 30 sampel pasien LLA anak dengan demam neutropenia di RSUD Dr Soetomo Juni 2018-Juni*

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*2020 didapatkan 17 pasien (56.7%) memiliki kategori skor risiko sedang, dan 13 lainnya berada dalam kategori risiko rendah (43.3%). Pasien didominasi oleh neutropenia derajat sedang dan berat masing-masing 43.3%, mengalami neutropenia selama 1-7 hari (50%), demam kurang dari tujuh hari (66.7%), memiliki lama rawat 8-14 hari, dan 15- 30 hari masing-masing 33.3%. Kesimpulan dari penelitian ini ada hubungan yang bermakna antara kategori skor Rondinelli pada pasien ALL pediatrik dengan FN dengan keparahan neutropenia p=0.037; R=0.383), lama neutropenia (p=0.021; R=0.420), lama demam (p=0.000; R=0.618), dan lama rawat inap (p=0.005; R=0.496).*

***Kata kunci:*** *Demam neutropenia; leukemia limfoblastik akut; kanker; skor Rondinelli; infeksi*

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## INTRODUCTION

Febrile neutropenia (FN) is when axillary temperature >38ºC persists for more than two hours or axillary temperature >39ºC on one measurement with neutrophils <1000 cells/ microliter.[1] Febrile neutropenia is the most severe complication that accounts for 50%-70% of deaths in patients with hematological malignancies and chemotherapy.[2] Acute lymphoblastic leukemia is the most common type of acute leukemia in children. At RSUD Dr. Soetomo Surabaya, acute leukemia was in the first rank of malignancy patients in children within a period of 10 years (1991-2000) which is 59% of all malignancies in children.[3] ALL is also the most common cause of FN cases in Sanglah Hospital, which is 33.3%.[4] In leukemia patients without chemotherapy, neutrophils will actually decrease significantly by themselves through the direct interaction of cancer with hematopoiesis. However, if the patient is undergoing chemotherapy, the interaction of several cancer drugs also has a myelosuppressive effect that interferes with the formation of neutrophils.[2] This makes ALL patients very susceptible to febrile neutropenia due to systemic bacterial infection or severe sepsis, which can be life-threatening.[5]

The life-threatening nature of febrile neutropenia (FN) makes an initial risk assessment necessary to increase clinicians' alertness.[2] For patients with low risk, this risk stratification can provide benefits in the form of improving quality of life, reducing the risk of exposure to nosocomial infections in hospitals, and can also reduce the burden of treatment costs.[6] The Rondinelli scoring system is a reasonably good instrument for predicting severe infectious complications in pediatric patients with ALL who have febrile neutropenia.[7] FN patients will be categorized into low risk, medium risk, or high risk using several parameters: age, hemoglobin, central venous catheter access, location of focal infection, fever temperature, and the emergence of upper respiratory tract infection (URTI).[8]

Clinical outcomes in the form of the severity of neutropenia, duration of neutropenia, duration of fever, and length of stay were recorded to see clinical improvement in FN patients after empiric antibiotic therapy. This study aims to determine the relationship between the risk category for febrile neutropenia (FN) based on the Rondinelli score with clinical outcomes in FN patients with acute lymphoblastic leukemia (ALL) in the Hematology-Oncology division of the child health department of RSUD Dr. Soetomo.

## MATERIALS AND METHODS

### Materials

The collected data is processed and grouped according to the Rondinelli scoring criteria as shown in Table 1.

**Table 1.** Rondinelli Scoring Criteria

| Clinical Variables | Score |
|---|---|
| Age ≤ 5 years | 1 |
| Age > 5 years | 0 |
| Central venous catheter | 2 |
| Without central venous catheter | 0 |
| Focal infection | 4,5 |
| Without focal infection | 0 |
| Fever ≥ 38,5 ºC | 1 |
| Fever < 38,5 ºC | 0 |
| Hemoglobin ≤ 7 g/dl | 1 |
| Hemoglobin >7 g/dl | 0 |
| Without URTI | 0 |
| URTI | 2,5 |

Patients are categorized as low risk if the total score is <5.5, moderate risk if the score is 5.5 to 9, and more than 9 is high risk.[1]

## Methods

This study is a retrospective observational analytic study using secondary data of FN patients with acute lymphoblastic leukemia (ALL) in the Hematology-Oncology division of the child health department of RSUD Dr. Soetomo June 2018-June 2020. This research has met the requirements of ethical feasibility by the Ethics Committee RSUD Dr. Soetomo, Surabaya (0333/LOE/301.4.2/II/2021). Sampling is done by total sampling. The inclusion criteria for this study were medical records of pediatric patients (0-18 years) with ALL who were diagnosed with complications of febrile neutropenia in the complete daily records of the doctor in charge of hematology-oncology. Incomplete medical record data were excluded from this study. This research analysis uses the IBM SPSS STATISTICS version 24 application using the Crosstabs technique and Spearman's test for non-parametric data.

## RESULTS AND DISCUSSION

There is no FN ICD (The International Classification of Diseases) in the electronic data center, so the authors selected the inclusion data in two stages. The first stage, looking for medical record numbers with ICD ALL and ICD neutropenia at the electronic medical record data center, obtained 90 medical records. In the second stage, from the 90 medical records, data were taken with the diagnosis of FN complications in printed medical records, and obtained 30 medical record data.

### Sample Characteristic FN Patients with LLA

**Table 2.** Sample Characteristic

| Variables | Category | N=30 | Percentage (%) |
|---|---|---|---|
| Gender | Male | 17 | 56.7 |
| | Female | 13 | 43.3 |
| Age (year) | >5 | 17 | 56.7 |
| | ≤5 | 13 | 43.3 |
| Hemoglobin (g/dL) | >7 | 29 | 96.7 |
| | ≤7 | 1 | 3.3 |
| Central venous catheter | No | 30 | 100.0 |
| | Yes | 0 | 0.00 |
| Focal infection | No | 10 | 33.3 |
| | Yes | 20 | 66.7 |
| Temperature (°C) | <38.5 | 21 | 70 |
| | ≥38.5 | 9 | 30 |
| URTI | No | 23 | 76.7 |
| | Yes | 7 | 23.3 |
| Rondinelli score category | Low | 13 | 43.3 |
| | Moderate | 17 | 56.7 |
| | High | 0 | 0 |

From Table 2, Pediatric ALL patients with febrile neutropenia were 17 male (56.7%) and 13 female (43.3%). This study were similar to previous studies that pediatric ALL patients with febrile neutropenia were predominantly male.[1,7,8,9] Most patients aged over five years, as many as 17 people (56.7%) in line with the previous study that patients aged above 5 years more in number than under 5 years,[1,8] but the proportion is almost the same.[7] This study is similar to the previous studies in that patient's hemoglobin level was predominantly above 7

g/dL.[1,8] From table 2, similar to the previous findings that none of the pediatric ALL patients who had FN used a central venous catheter.[1,7] This study found most patients have a specific focus of infection same like previous studies.[1,8] In this study, most patients had a subfebrile temperature that was below 38.5°C with a percentage similar to the results of Rondinelli et al.[8], which was 70% vs 65%. However, other studies show the opposite, the patient's temperature is mostly above 38.5°C with a percentage of more than 60%.[1] Some febrile neutropenic patients whose temperature was recorded as normal in this study may be due to the author's error in entering the data, namely choosing which fever temperature corresponds to the time when the patient was diagnosed with febrile neutropenia. Another cause was that the patient had no fever when he checked his vital signs by a hematologist-oncologist. From this study, patients were found to have upper respiratory tract infections (URTI) only about 10%-20%.[1,8]

Based on the calculation rules of the Rondinelli score system from Table 2, pediatric patients who experienced neutropenic fever at Dr. Soetomo Hospital for the period June 2018-June 2020, 17 patients (56.7%) had a moderate risk score category, and 13 others had a low-risk category (43.3 %). This study is different from previous studies because there were no high-risk patients (score > 9.0). According to the research conducted by Andrieanta et al.[1] at Dr. Cipto Mangunkusumo Hospital, Jakarta of the 96 samples of patients with febrile neutropenia, 52% had a low category, 48% had a high category, and none had a moderate category.[1] In another study conducted by Hidayat et al. of 30 samples, 73.33% had low risk (score <5.5), and the rest (score 5.5) as many as 26.67% had moderate and high risk.[7] Previous studies have shown varied results regarding the distribution of the risk level of FN patients with Rondinelli scores. So it can be concluded that there is no absolute prevalence rate related to the risk of FN patients based on the Rondinelli score, depending on the situation in each hospital, and further research is needed to find out this.

## Clinical Outcome FN Patients with LLA

**Table 3.** Result Of Clinical Outcome

| Variables | Category | N=30 | Percentage (%) |
|---|---|---|---|
| **Severity of neutropenia** | Low | 4 | 13.3 |
| | Moderate | 13 | 43.3 |
| | Severe | 13 | 43.3 |
| **Length of neutropenia (days)** | 1-7 | 15 | 50 |
| | 8-14 | 9 | 30 |
| | 15-25 | 6 | 20 |
| **Length of fever (days)** | ≤7 | 20 | 66.7 |
| | >7 | 10 | 33.3 |
| **Length of hospitalization (days)** | 1-3 | 1 | 3.3 |
| | 4-7 | 7 | 23.3 |
| | 8-14 | 10 | 33.3 |
| | 15-30 | 10 | 33.3 |
| | >30 | 2 | 6.7 |

Based on Table 3 the findings of this study were dominated by the severity of moderate and severe neutropenia, respectively 43.3%. Similar to the previous study, which found that malignancy patients with FN were dominated by the severity of severe neutropenia as much as 55.55%.[10] This study found that the duration of ALL patients with FN was in line with the previous finding, which was dominated by the duration of neutropenia of less than 7 days.[9]

The results of this study also showed that most febrile neutropenic patients had fever for less than seven days, which was 66.7%, almost the same as the previous study, which was 80.8%.[11] Based on Table 3, patients mostly had duration of hospitalization for 8-14 days and 15-30 days, respectively 33.3%. This is in line with previous studies which found that the longest duration of hospitalization for patients was 15-30 days.[9]

**Table 4.** Correlation Rondinelli Score with Clinical Outcomes

| Risk Category | p-value | R |
|---|---|---|
| Length of Hospitalization | .005 | .496** |
| Length of Fever | .000 | .618** |
| Length of Neutropenia | .021 | .420* |
| Severity of Neutropenia | .037 | .383* |

Bivariate analysis using Spearman's test

**Correlation Rondinelli Score with Severity of Neutropenia**

Based on Table 4, there is a moderate, positive, and significant correlation between the FN risk score category and the severity of neutropenia with p=0.037 (p<0.05) and R= 0.383, which means that the higher the Rondinelli score, the greater the risk of FN patients experiencing infectious disease complications, the more severe the severity of neutropenia will be. In cases of neutropenia with infectious complications, especially mucositis, and gastrointestinal infections, the patients' ANC showed a significantly lower ANC.[12] This is in line with the findings of Badiei et al. that ANC <100 is one of five variables that have a significant correlation with the incidence of life-threatening infections.[13] The other four predictor variables were fever, mucositis, platelet count, and abnormal lung temperature. This variable is very similar to the component of the Rondinelli score that assesses the risk of infectious complications in febrile neutropenic patients.[13] Furthermore, from another study, the high risk group with the MASCC score that had an ANC <500 showed a larger number of patients, namely 55% compared to low risk patients, which was only 45%.[14] Another study showed that the incidence of infection in the ANC count <500 was much higher than the ANC between 500-1000, which was 71.4% compared to 28.6%.[15] Thus, it can be concluded that there is a significant relationship between the Rondinelli score category and the severity of neutropenia, related to the incidence of infection. This is because neutrophils are cells that fight and destroy pathogens that cause infection once they are in circulation.[16]

**Correlation Rondinelli Score with Length of Neutropenia**

Based on Table 4, there is a moderate and unidirectional significant relationship between the FN risk score category and the duration of neutropenia with p=0.021 (p<0.05) and R=0.420, which means that the higher the Rondinelli score, the greater the risk of FN patients experiencing infectious complications, the longer the duration of neutropenia. The same result was obtained from another study in that there was a significant relationship (p = 0.046) between the duration of neutropenia and the risk level of pediatric patients with FN.[17] Likewise from Alexander et al. who said that high-risk FN patients had a significantly longer duration of neutropenia than low-risk patients. This is because the longer the patient experiences neutropenia, the higher the risk of the patient experiencing infection due to the weakening of the patient's body defenses against infectious agents.[18] The Guidelines of the Infectious Diseases Working Party, Germany has previously classified the risk of infection based on the duration of the patient experiencing neutropenia. It is said to be low risk if the duration of neutropenia is less than five days, intermediate risk if the duration of neutropenia is 6-9 days, and high risk if the duration of neutropenia is at least 10, days.[19]

**Correlation pf Rondinelli Score with Length of Fever**

Based on Table 4, there is a strong, positive, and significant correlation between the FN risk score category and the duration of fever with p=0.000 (p<0.0001) and R=0.618. It means that the higher the Rondinelli score, the greater the risk of FN patients experiencing infectious complications, the longer the duration of the patient's fever. The results of this study are in line with previous findings which stated that the high risk group of pediatric patients with febrile neutropenia had a significantly longer duration of fever (p < 0.001) than the low risk group.[17] Another study said that the median duration of fever in pediatric patients with FN in the group with blood stream infection was significantly greater than in the group without infection.[20] This is because fever is an indication of infection, which accounts for a mortality rate of 1%-3% in pediatric patients with all who are undergoing chemotherapy.[21] In 20-30% of pediatric cancer patients with febrile neutropenia due to bacterial infection, other causes are viral infections, blood product transfusions, cytostatic drugs, malignancy itself, and mucositis.[17]

**Correlation Rondinelli Score with Length of Hospitalization**

Based on Table 4, there is a fairly strong and unidirectional significant relationship between the FN risk score category and the length of stay with p=0.005 (p<0.05) and R=0.496, which means that the higher the Rondinelli score, the greater the risk of FN patients experiencing infectious complications, the longer the duration of patient care. In line with another study using the Clinical Index of Stable Febrile Neutropenia (CISNE) score, it was said that the increase in the risk category of FN patients was directly proportional to the duration of hospital stay (p <0.001).[14] Likewise, the Multinational Association for Supportive Care in Cancer (MASCC) score system shows a longer average duration of care in the high risk group than the low risk group.[22] Longer duration of hospitalization in pediatric patients with high-risk FN was also found in another study.[23] Complications such as bacteremia/sepsis, pneumonia, bacterial or fungal infections significantly increase the duration of hospitalization for pediatric patients with FN.[24] Another study also showed that the median duration of stay of patients with blood stream infection (BSI) was found to be longer than that of patients without an identified pathogen (19 days versus 10 days).[25] Likewise, patients with MDI (microbiologically documented infection) were said to be 4.5 times more likely to have a prolonged duration of stay.[26] The duration of hospitalization for pediatric FN patients also had a significant relationship with the incidence of septicemia (p <0.0001).[27]

## CONCLUSIONS

This study found that pediatric ALL patients who experienced febrile neutropenia in the Hematology-Oncology division of the child health department of RSUD Dr. Soetomo most of them have medium risk category and the rest are low risk. There is a fairly strong and direct significant relationship between the Rondinelli score category and the severity of neutropenia, duration of neutropenia, and length of stay. There is also a strong and direct significant relationship between the Rondinelli score category and the duration of fever. Thus it can be concluded that the higher the Rondinelli score, the more severe the severity of the patient's neutropenia, the longer the patient's duration of treatment, the longer the patient's fever, and the longer the patient's neutropenia.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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