

OCCURRENCE OF COVID-19 IN CHILDREN WHO HAVE RECEIVED LIVE-ATTENUATED DENGUE VACCINATION

Mulya Rahma Karyanti^{1*}, Anggraini Alam², Indah Suci Widyahening³, Sri Rezeki Hadinegoro¹, Zakiudin Munasir¹, R. Tedjo Sasmono⁴, Hindra Irawan Satari¹

¹ Department of Child Health, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia; ² Medical Faculty University Padjajaran, Hasan Sadikin hospital, Bandung, Indonesia; ³ Department of Community Medicine, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia; ⁴ Eijkman Research Center for Molecular Biology, National Research and Innovation Agency, Indonesia.

Corresponding Author

Name : Mulya Rahma Karyanti
Email : karyanti@ikafkui.net
Mailing address : Departement Ilmu Kesehatan Anak, RSCM FKUI
Telephone : +62811963885
Fax : +62213907742
ORCID ID : 0000-0002-7194-9403

Abstract

Background: Possible cross-reactions/ false positives were reported in rapid dengue serology tests because antigenic similarities between SARS-CoV-2 and DENV. The study aims to evaluate the occurrence of COVID-19 in children who received chimeric-yellow fever tetravalent dengue vaccine. **Methods:** A case-control study was conducted in five districts primary healthcares in Jakarta aged 12 years and above through history of COVID-19 questionnaire. Clinical and laboratory confirmation were obtained, and blood draw was performed to measure neutralization RBD IgG SARS-CoV-2 antibody titer. The dengue vaccine group consists of subjects who have received CYD-TDV in 2011-2012 and are willing to participate. The non-dengue vaccine group were matched; all have not received dengue vaccine. **Results:** This study included 207 cases and 212 controls, with median age in cases 19 years (IQR 5) and control 15 years (IQR 4). Nineteen subjects in the dengue vaccine group have already been infected with COVID-19 before being given COVID-19 vaccine, compared to 11 subjects in the non-dengue vaccine group ($P=0.131$). The occurrence of COVID-19 in the dengue vaccine group was significantly higher (16 subjects) than the non-dengue vaccine group (4 subjects) ($P=0.005$) after COVID-19 vaccinations were given. Neutralization RBD IgG SARS-CoV-2 antibody titer was 71.96 U/ml (IQR 39.47) in the dengue vaccine group and 51.92 U/ml (IQR 49.03) in the non-dengue vaccine group ($P=0.361$). **Conclusion:** Our study showed that the occurrence of COVID-19 in the dengue vaccine group was higher than in the non-dengue vaccine group, which may suggest no cross reaction from dengue antibodies towards COVID-19, more studies are warranted.

Keywords

COVID-19, dengue vaccine, children, cross-reaction, dengue infection

Introduction

The coronavirus disease 2019 (COVID-19) pandemic, causing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was an international public health emergency concern that causes double burden in tropical and subtropical countries where dengue is endemic. Indonesia, being a highly endemic dengue country, has also faced COVID-19 pandemic since March 2020, which further adds the national healthcare burden.¹ The reported COVID-19 cases continued to rise; from two confirmed COVID-19 cases reported in March 2, 2020 until April 20, 2022 reach 2.15 per 100,000 population per week. Data from the Ministry of Health's Directorate General of Disease Prevention and Control 2022, reported increase dengue hemorrhagic fever (DHF) cases from 2011 to 2022. In 2019 IR of DHF reach 51.1 per 100,000 population and gradually decrease until 2022 to IR of DHF 6.8 per 100,000 population.²

Countries with a high dengue endemicity have less COVID-19 incidence, transmission, and mortality. Based on the non-overlap countries with dengue and COVID-19, and the increasing evidence of SARS-CoV-2 false-positivity in dengue antibody tests, speculation whether pre-exposure to dengue virus (DENV) in highly endemic countries may provide protection against COVID-19 become a question. Whether immunization of susceptible populations with available live-attenuated dengue vaccines will prompt the anti-viral immune response to prevent COVID-19.³

Diagnostic challenges occurred as the clinical manifestations of COVID-19 and dengue are similar and the emerging concern of false positive results due to cross-reactivity of the immune responses in these infections.¹ Cross-reactions of antibody test between SARS-CoV-2 and dengue were reported in several countries.⁴⁻⁶ Coinfection of dengue and COVID-19 was reported in several dengue-endemic countries,⁷⁻¹⁰ including Indonesia.^{5, 11}

A study reported low risk of serological cross-reactivity between dengue and COVID-19. From 32 COVID-19 positive sera, no positive DENV IgG/IgM results were found. Only one false-positive result was detected among 44 DENV-positive sera tested for COVID-19 antibodies. The accuracy of COVID-19 diagnostic tests need to be explored further.¹²

A study in Brazil showed that in 2351 patients with COVID-19, 50% reported previously having dengue infection. Those without previous dengue infection had a higher risk of death (hazard ratio: 0.44; 95% confidence interval: 0.22-0.89; $p=0.023$) in 60-day follow-up. This finding raises concern that there is a possibility that dengue induces immunological protection against COVID-19.¹³

In 2011, Sanofi Pasteur conducted a live attenuated dengue vaccine research, consisting of chimeric yellow fever tetravalent dengue vaccine (CYD-TDV), which has

progressed to phase III of clinical trials in five countries in Asia (Indonesia, Malaysia, Singapore, the Philippines, and Vietnam) and five countries in Latin America (Colombia, Brazil, Mexico, Puerto Rico and Honduras). The vaccine's efficacy in the age of 9 years or more could prevent the incidence of severe dengue by 93.2% and prevent hospitalization due to dengue infection by 80.8%.¹⁴ The efficacy of the dengue vaccine after 6 years shows strong protection in preventing hospitalization and virologically confirmed severe dengue at ages nine years or more and ages 6-8 years who are seropositive.¹⁵

This study aims to evaluate the occurrence of COVID-19 with the clinical manifestations in children who have received the chimeric yellow fever tetravalent dengue vaccine (CYD-TDV) vaccine compared to children who have not received the CYD-TDV vaccine. Moreover, this study evaluates the difference in SARS-CoV-2 receptor-binding domain (RBD) neutralizing antibody titer between the dengue vaccine group and the non-dengue vaccine group.

Materials and Methods

Study design, patient' recruitment, and ethical approval.

This study was part of the CYD-TDV effectiveness study after 10 years in Indonesia during the COVID-19 pandemic. In Indonesia, the mandatory nation-wide COVID-19 vaccinations for children aged 12-17 years were given in June 2021. The dengue vaccine group consisted of subjects who received CYD-TDV vaccinations ten years ago. In contrast, the control group (non-dengue vaccine group) are subjects who have not received dengue vaccination.

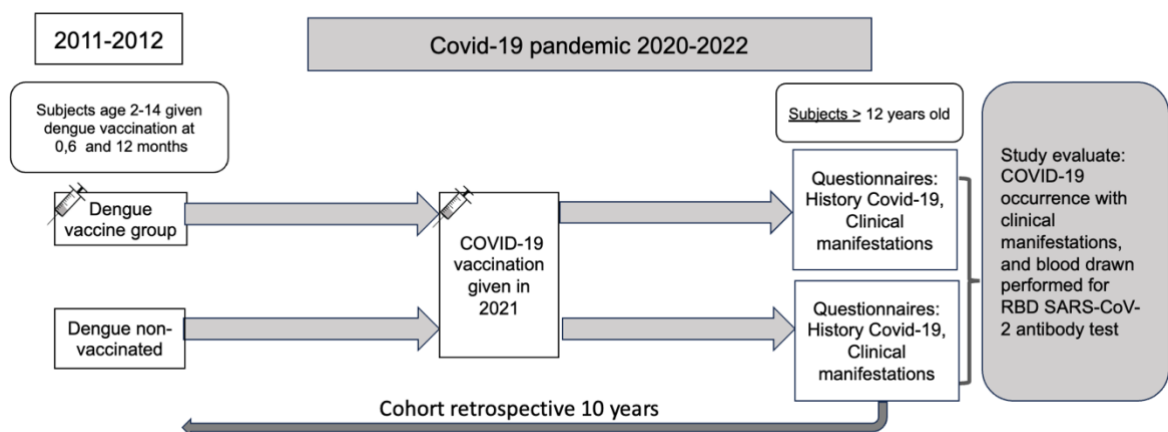


Figure 1. Scheme of the research protocol

The methodology design of this study was a retrospective cohort, evaluating the history and obtaining clinical signs and symptoms of COVID-19 in the dengue

vaccine group compared to the non-vaccine group with questionnaires and confirming the results from the healthcare facilities where the subject was diagnosed COVID-19 as outpatient or inpatient before and after given national program COVID-19 vaccination in 2021. The type of COVID-19 vaccine received will be recorded in the database. A cross-sectional study was also performed to evaluate the SARS-CoV-2 IgG antibody qualitative test and investigate the difference in titer of neutralization antibody RBD SARS-CoV-2 between the dengue vaccine group and the control group.

Inclusion criteria were healthy subjects who had received the CYD-TDV dengue vaccine three times in 2011 - 2012, and lived in DKI Jakarta province. Meanwhile, those in the control group were healthy subjects who had not received the dengue vaccine, aged 12 - 18 years, both male and female, from the same one sub-district or one school as those in the dengue vaccine group, and that their parents agreed to participate in this study. Exclusion criteria were subjects who suffered from an acute infection within seven days before research recruitment began or for subjects who had been diagnosed with an immune system disorder (immunocompromise) such as HIV, primary immunodeficiency or received long-term corticosteroid treatment (more than two weeks) or that the subject's address cannot be found.

The study was conducted in five district primary health centers in Jakarta. All subjects completed questionnaires asking about their history of COVID-19, clinical and laboratory confirmation were obtained from the corresponding hospital admission, and blood draw was performed in a subset to measure the titer of IgG antibody neutralization against the SARS-CoV-2 RBD antigen. The ethical approval was obtained from The Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Number KET-465/UN2.F1/ETIK/PPM.00.02/2022, and also was approved by the Provincial Public Health Office of DKI Jakarta, Number 4855/TM.09.4.

Statistical Analysis

Data was analyzed using the two-tailed Mann-Whitney test with SPSS version 24 to assess the difference between the dengue vaccine group and the non-vaccine dengue group. Meanwhile, to measure the dichotomous variable of SARS-CoV-2 IgG antibody, a Chi-square test and Fisher's exact test will be performed and compare RBD SARS-CoV-2 antibody titer using a non-parametric test between the two groups.

Results

There were 419 subjects in total who fulfilled the inclusion criteria, consisting of 207 subjects in the dengue vaccine group and 212 subjects in the non-dengue vaccine group (control), with a total of 79 subjects in the subset of the dengue vaccine group, and 80 subjects in the control group.

The age of the dengue vaccine group and non-dengue vaccine group data shows that the median age of the dengue vaccine group was 19 (IQR 5) years old, while the non-dengue vaccine group had a median age of 15 years (IQR 4). The dengue vaccine and non-vaccine groups were 54.4% in normal nutritional status. The occurrence of COVID-19 in the dengue vaccine group was higher than in the non-dengue vaccine group before COVID-19 vaccination, although statistical analysis was not significantly different (Table 1). Meanwhile, the incidence of COVID-19 in the dengue vaccine group was significantly higher ($p=0.005$) than the non-dengue vaccine group after COVID-19 vaccination. The hospitalization and outpatient care due to COVID-19 infection in both groups were not significantly different before or after the COVID-19 vaccination. The degree of COVID-19 in the dengue and non-dengue vaccine groups was dominated by mild degrees. Meanwhile, there was no significant difference in the length of stay (LOS) for COVID-19 in the vaccine and non-dengue vaccine groups (Table 1).

Table 1. COVID-19 occurrence and the degree of COVID-19

Before COVID-19 vaccination	Dengue-Vaccine (n=207)	Non-Dengue Vaccine (n=212)	P value	After COVID-19 vaccination	Dengue-Vaccine (n=207)	Non-Dengue Vaccine (n=212)	P value
COVID-19 positive	19	11	0.113	COVID-19 positive	16	4	0.005
• Outpatient	7	8	0.128	• Outpatient	15	4	1.000
• Inpatient	12	3		• Inpatient	1	0	
COVID-19 negative	188	201		COVID-19 negative	191	208	
Degree of COVID-19				Degree of COVID-19			
• No symptom	2	2		• No symptom	4	2	
• Mild	13	7		• Mild	10	2	
• Moderate	4	2		• Moderate	2	0	
Length of stay COVID-19	14 (2-17)	7 (3-13)	0.107	Length of stay COVID-19	9	0	

The incidence of COVID-19 in the dengue vaccine group was higher in the vaccine group than in the non-dengue vaccine group based on year stratification during the 2020-2022 pandemic (Figure 2).

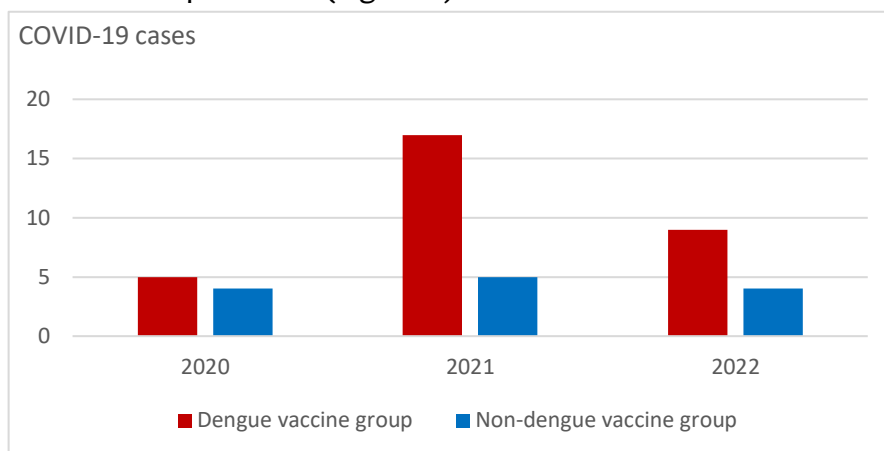


Figure 2. The incidence of COVID-19 in vaccine-dengue group vs non-vaccine dengue group based on annual incidence during pandemic.

The most frequently signs and symptoms of COVID-19 before the COVID-19 vaccine in the dengue vaccinated group were anosmia, followed by ageusia (loss sense of taste), fever and cough. Meanwhile, those in the dengue non-vaccinated group had coughing, nasal congestion (blocked nose), and anosmia (Figure 3.)

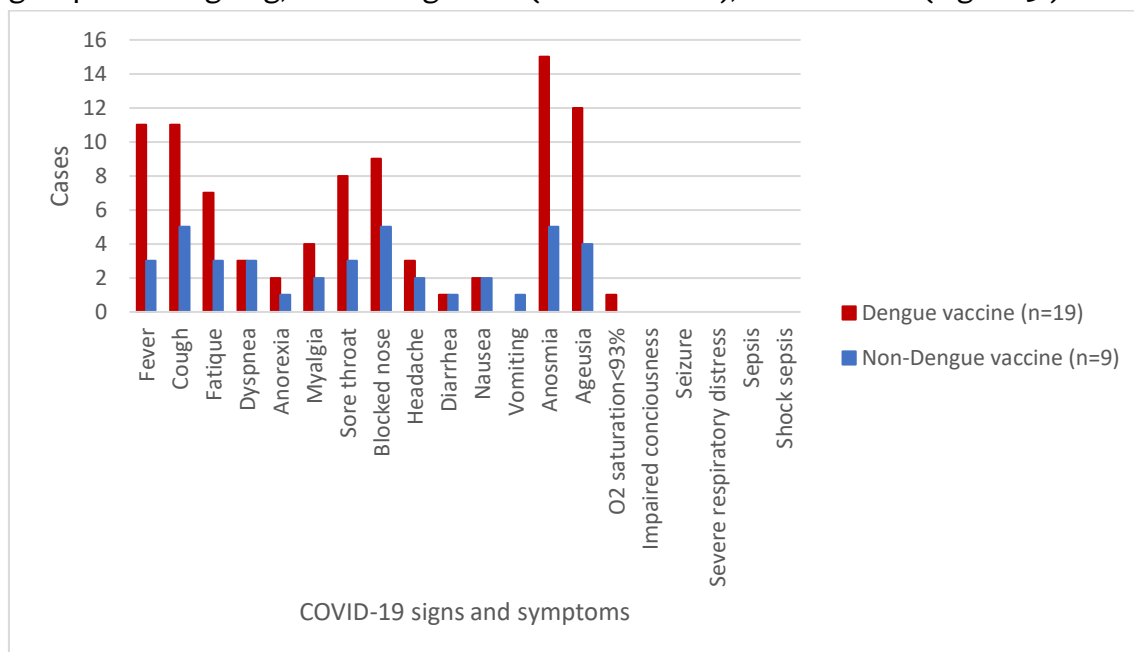


Figure 3. Signs and symptoms of COVID-19 before receiving COVID-19 vaccination.

Figure 4 shows that the most common signs and symptoms of COVID-19 after the COVID-19 vaccine in the dengue vaccine group were fever, cough, blocked nose,

and headache. Meanwhile, the most common symptoms in the non-dengue vaccine group were sore throat and headache.

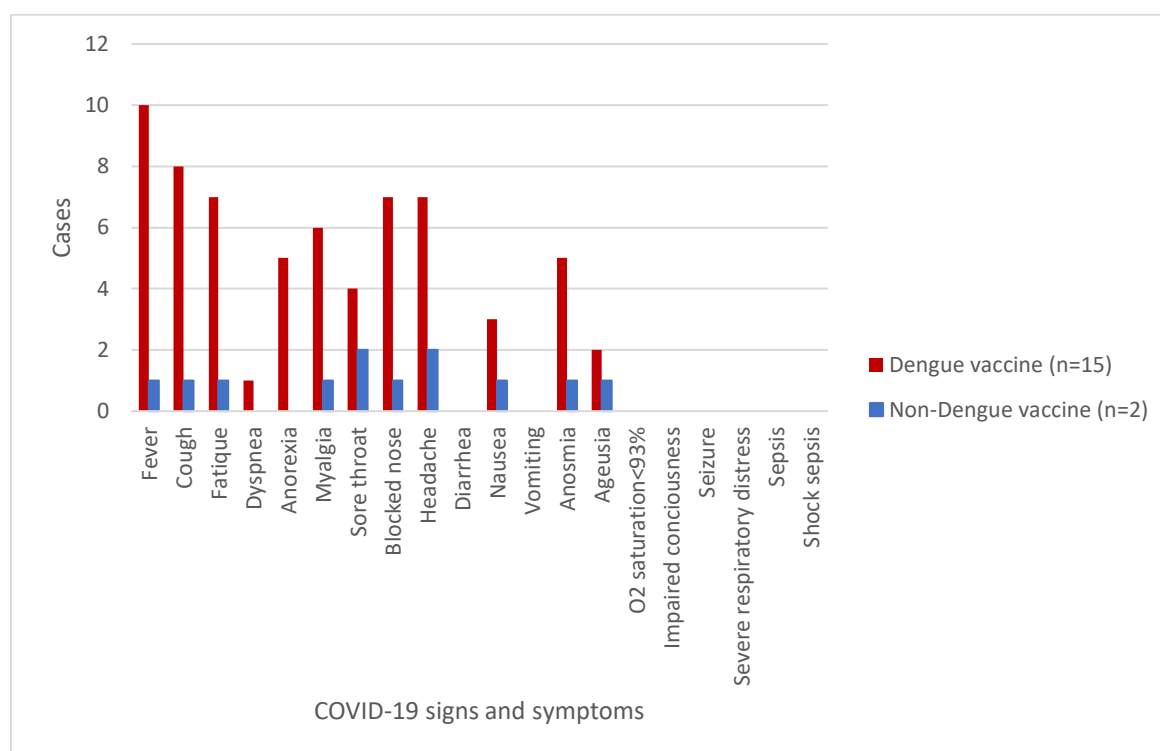


Figure 4. Signs and Symptoms of COVID-19 after receiving COVID-19 vaccination

This study showed that the antibody profile for SARS-CoV-2 in those who did not receive the COVID-19 vaccination between the dengue and non-dengue vaccine groups was not significantly different. Nonetheless, the titer of neutralizing SARS-CoV-2 IgG antibodies in the dengue vaccine group was higher than the non-dengue vaccine group with a value of 71.96 U/ml (IQR 39.47) vs. 51.92 U/ml (IQR 49.03). However, they were not statistically significant ($P=0.361$).

Discussion

There are several reports of cross-reactions between dengue antigens and antibodies with COVID-19. A study suggested a possibility of seroprotection from past dengue infection to SARS-CoV-2 infection. However, there is still a lack of evidence, especially serologically, whether post-dengue vaccination or history of dengue infection could protect against SARS-CoV-2 infection.^{10, 16} In our study, we found that the occurrence of COVID-19 in the dengue vaccine group was higher than that in the non-dengue vaccine group before COVID-19 vaccination. Meanwhile, the incidence of COVID-19 in the dengue vaccine group was significantly higher ($p=0.005$) than the non-dengue vaccine group after COVID-19 vaccination.

Our study showed that subjects in the dengue vaccine group who already have had tetravalent dengue antibodies had higher COVID-19 infection rate than in the non-dengue vaccine group, suggesting that there is no protection from the dengue antibody against SARS-CoV-2 infection. Despite that, the titer of neutralizing SARS-CoV-2 IgG antibodies in the dengue vaccine group was higher than that in the non-dengue vaccine group, albeit with no significant difference ($p=0.361$). We assume that the dengue vaccine group might be exposed to SARS-CoV-2 more often than the non-vaccine group.

Several reports have shown that *in vitro*, there were false positive results and cross-reaction from IgG and IgM rapid diagnostic serology test for dengue with SARS-CoV-2 IgG and IgM antibodies, and vice versa.^{4, 5} Furthermore, there is another study using computational model reported that dengue antibodies reduce the severity of COVID-19. This computational modeling research showed that dengue antibodies reduce the severity of COVID-19 and the spread of COVID-19. It is postulated that dengue antibodies bind to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, and there is potential for interception of human ACE2 receptor binding with the receptor binding motif (RBM). Therefore, dengue antibodies have the potential to compete for the ACE2 receptor, which is the RBD access from SARS-CoV-2. Based on *in-silico* analysis research, it was found that there are similarities between the epitope in the Heptad Repeat (HR)2 domain of the spike protein and the dengue envelope protein, so it is possible for a cross-reaction to occur between the dengue virus and SARS-CoV-2, which could result as false positives of dengue serology in COVID-19 patients and vice versa.¹⁶ Cross-reaction between dengue and COVID-19 antibodies in dengue rapid diagnostic test (RDT) has been reported in 2 patients with confirmed COVID-19 in Singapore.¹⁷ Santoso et al. reported cross-reaction and false-positive results between dengue and COVID-19 by assessing 95 RT-PCR-confirmed COVID cases using dengue serology antibody tests, detected only one IgM-positive case.⁶

Our study showed that *in vivo*, the dengue vaccine group had higher rates of symptomatic mild COVID-19 infections than the non-dengue vaccine group. This is supported by an *in vitro* study reporting no positivity of the ELISA IgM/IgG test for SARS-CoV-2 in the serum samples of dengue patients.^{4, 5, 10} However, another study by Spinicci et al. showed that there was no IgM/IgG positivity in ELISA test for dengue found in patients with COVID-19, making the risk of cross-reactivity between dengue and SARS-CoV-2 low.¹²

Meanwhile, Silvestre et al. reported that cases with a history of dengue infection have lower mortality from COVID-19. Nonetheless, the study could not describe the causal association between previous dengue and immunity-improving prognosis from SARS-CoV-2 infection and therefore suggest further studies.^{13, 18} Our study did not show any mortality due to SARS-CoV-2, as all the majority of COVID-19

infections occurred in both groups were mild COVID-19 infections. The cause of the difference in the incidence could be attributed to differences in the COVID-19 vaccination coverage, the variants of SARS-CoV-2 that were circulating, and the age of the population.

Countries with high dengue endemicity showed lower COVID-19 regarding infection, transmission, and mortality.³ Other ecological studies showed that from 22 major dengue epidemic countries, the incidence of dengue infections during the pre-COVID-19 (2015-2019) decreased by 16% significantly ($p < 0.05$) compared to the COVID-19 period (2020-2021). Nonetheless, more comprehensive and evidence-based scientific approaches need to be conducted at all levels.¹⁹

The intensity levels and forms of mobility restrictions applied across different countries varied and temporally adapted in the months of 2020. This provides a unique opportunity to analyze these variables in how they affect transmission in infectious diseases other than COVID-19, such as dengue. As a dengue endemic country, Indonesia was hardly affected by COVID-19 in 2020. The government imposed a restriction policy in early March 2020 that was not a full lockdown, but instead a compromise between transmission control and economic considerations, called 'large-scale social restriction', which decreases human movement by promoting work-at-home and school closures to prevent the spread of COVID-19 infection. The healthcare system might be more focused on the COVID-19 pandemic, which may reduce the intervention on surveillance and vector control in dengue-endemic areas.²⁰ More studies in the future are warranted to explore the reasons for differences in COVID-19 incidence and severity in various populations in different geographical dengue endemic regions.

Conclusions

Our study suggests that the incidence of COVID-19 infections is higher in the dengue vaccine group than in the non-vaccine group. However, most of the subjects from the dengue vaccine group only showed a mild degree of COVID-19 infections. This implies that children who have received dengue vaccines are not protected from COVID-19, suggesting that dengue antibodies may have no cross-reaction towards COVID-19.

Competing Interests

None declared.

Acknowledgments

The author would like to thank all health professionals involved in this study.

References

1. Harapan H, Ryan M, Yohan B, Abidin RS, Nainu F, Rakib A, et al. Covid-19 and dengue: Double punches for dengue-endemic countries in Asia. *Rev Med Virol.* 2021;31:e2161-8.
2. Triastusti NJ. The Comparison of Dengue Haemorrhagic Fever Cases in Indonesia During the COVID-19 Pandemic. *J Med Chem Sci.* 2023;6:1336-43.
3. Biswas S, Sukla S. COVID-19 virus infection and transmission are observably less in highly dengue-endemic countries: is pre-exposure to dengue virus protective against COVID-19 severity and mortality? Will the reverse scenario be true? *CEI* [Internet]. 2020 June 30. Available from: <http://dx.doi.org/10.31487/j.CEI.2020.02.05>.
4. Luhulima D, Soetowo T, Amelia R. Cross-Reaction Antibody Test between SARS-CoV-2 and Dengue Hemorrhagic Fever in Indonesia. *Indonesian Journal of Clinical Pathology and Medical Laboratory.* 2021;27(2):224-7.
5. Masyeni S, Santoso MS, Widyarningsih PD, Asmara DW, Nainu F, Harapan H, et al. Serological cross-reaction and coinfection of dengue and COVID-19 in Asia: Experience from Indonesia. 2021. p. 152-4.
6. Santoso MS, Masyeni S, Haryanto S, Yohan B, Hibberd ML, Sasmono RT. Assessment of dengue and COVID-19 antibody rapid diagnostic tests cross-reactivity in Indonesia. *Virol J.* 2021;18:54-8.
7. Epelboin L, Blonde R, Nacher M, Combe P, Collet L. COVID-19 and dengue co-infection in a returning traveller. *J Travel Med.* 2020;27:1-2.
8. Estofolete CF, Machado LF, Zini N, Luckemeyer GD, Moraes MM, Dos Santos TMIL, et al. Presentation of fatal stroke due to SARS-CoV-2 and dengue virus coinfection. *Journal of medical virology.* 2021;93(3):1770-5.
9. Verduyn M, Allou N, Gazaille V, Andre M, Desroche T, Jaffar M-C, et al. Co-infection of dengue and COVID-19: A case report. *PLOS Negl Trop Dis.* 2020;14:1-5.
10. Zubairy M, Kanani F, Vankwani S. Dengue and COVID-19 co-infection or cross-reactivity: retrospective study from a tertiary care hospital. *Arch Clin Microbio.* 2021;12:163-70.
11. Alam A, Sudarwati S, Hakim DDL, Mahdiani S. Case Report: Severe COVID-19 and Dengue in an Indonesian Infant. *Am J Trop Med Hyg.* 2021;104:1456-60.
12. Spinicci M, Bartoloni A, Mantella A, Zammarchi L, Rossolini GM, Antonelli A. Low risk of serological cross-reactivity between dengue and COVID-19. *Memorias do Instituto Oswaldo Cruz.* 2020;115:e200225-e.
13. Silvestre OM, Costa LR, Lopes BVR, Barbosa MR, Botelho KKP, Albuquerque KLC, et al. Previous Dengue Infection and Mortality in Coronavirus Disease 2019 (COVID-19). *Clin Infect Dis.* 2021;73:e1219--e21.
14. Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *NEJM.* 2015;373:1195-206.
15. Forrat R, Dayan GH, DiazGranados CA, Bonaparte M, Laot T, Capeding MR, et al. Analysis of Hospitalized and Severe Dengue Cases Over the 6 years of Follow-up

- of the Tetravalent Dengue Vaccine (CYD-TDV) Efficacy Trials in Asia and Latin America. *Clin Infect Dis*. 2021;73:1003-12.
16. Nath H, Mallick A, Roy S, Sukla S, Biswas S. Computational modelling supports that dengue virus envelope antibodies can bind to SARS-CoV-2 receptor binding sites: Is pre-exposure to dengue virus protective against COVID-19 severity? *Comput Struct Biotechnol J*. 2021;19:459-66.
 17. Yan G, Lee CK, Lam LTM, Yan B, Chua YX, Lim AYN, et al. Covert COVID-19 and false-positive dengue serology in Singapore. *Lancet Infect Dis*. 2020;20:536.
 18. Nicolete VC, Rodrigues PT, Johansen IC, Corder RM, Tonini J, Cardoso MA, et al. Interacting epidemics in Amazonian Brazil: prior dengue infection associated with increased Coronavirus disease 2019 (COVID-19) risk in a population-based cohort study. *Clin Infect Dis*. 2021;73:2045-54.
 19. Khan S, Akbar SMF, Yahiro T, Mahtab MA, Kimitsuki K, Hashimoto T, et al. Dengue Infections during COVID-19 Period: Reflection of Reality or Elusive Data Due to Effect of Pandemic. *Int J Environ Res Public Health*. 2022;19:1-12.
 20. Sasmono RT, Santoso MS. Movement dynamics: reduced dengue cases during the COVID-19 pandemic. *Lancet Infect Dis*. 2022;22:570-1.