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# Global Medical and Health Communication

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### RESEARCH ARTICLES

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Global Medical and Health Communication is a journal that publishes medical and health scientific articles published every 4 (four) months. Articles are original research that needs to be disseminated and written in English.

The submitted manuscript must be an article that has never been published, and the author must ensure that all co-authors have agreed by signing a statement on the seal. For original research, we accept the study which is last then 7 (seven) years when the manuscript is submitted. The manuscript is an original article free from plagiarism. When the article is published in another journal then in the next journal, the article will be disallowed.

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Zhang B, Kunde D, Tristram S. *Haemophilus haemolyticus* is infrequently misidentified as *Haemophilus influenzae* in diagnostic specimens in Australia. Diagn Microbiol Infect Dis. 2014;80(4): 272–3.

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Miller LG. Community-associated methicillin resistant *Staphylococcus aureus*. In: Weber JT, editor. Antimicrobial resistance. Beyond the breakpoint. Basel: Karger; 2010. p. 1–20.

**Conference Proceeding**
Nicholai T. Homeopathy. Proceedings of the Workshop Alternative Medicines; 2011 November 30; Brussels Belgium. Brussels: ENVI; 2011.

**Journal Article from Internet**
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**RESEARCH ARTICLE**

# Promotion of Crypt-like Structures in Intestinal Organoid Development through the Addition of Graphene Oxide in Cell-based Assays

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**Abstract**

The intestinal organoid represents a miniature organ that can mimic functional physiology and pathology. However, there are several challenges to developing the organoid system, such as the limited survival of cells. Based on theory, matrix addition is a factor that can support survival in cells. As a result, graphene oxide (GO) addition is used in this study. As an artificial matrix, GO has been successfully shown to encourage good cell behavior and is well known for having good biocompatibility. Herein, we fabricate GO characterized with FT-IR and PSA. Crypt-like structures (CLS) are isolated from small intestinal mice in GO addition as a matrix. The gene expression and cell viability of CLS are investigated. RT-PCR examined the gene expression in CLS, while cell viability of CLS was carried out using the staining method. This study was conducted at FiNder U-CoE and Parasitology Laboratory of HSE Universitas Padjadjaran Bandung during February and December 2023. Our results show that Vil-1 as an identity for cells in the intestinal epithelium has been expressed in CLS primary significantly higher than intestinal tissue (p=0.01). However, identifying Lgr5 in CSL isolates is tricky. Thes in the crypt may be limited. Besides that, cell viability of CLS with GO addition can be maintained for four days. The GO addition as a matrix may provide support to maintain CLS. These findings are promising as cell-based assays for developing organoid models.

**Keywords:** Cell culture, graphene oxide, matrix, organoid

## Introduction

Cell-based assays have been an important component of drug discovery because they provide a simple, quick, and cost-effective technique for avoiding large-scale and costly animal testing.[1] Two-dimensional (2D) cell cultures as traditional in vitro assays have been widely used in the pharmaceutical industry for drug discovery.[2] However, these assays have several obstacles, such as not being able to control cell shape, losing the ability to regulate cells, being limited to a single type of cell, and not being able to reflect the physiological complexity of the tissue so that it can produce bias in predicting specific tissue responses.[1,3] Recently, three-dimensional (3D) cell cultures have been developed as a better model for evaluating and promoting improved cell- and organ-based assays for representative physiology and better drug response prediction.[4,5] The intestinal organoid is one of the most commonly used models. Previous studies have shown that the intestinal organoid can recapitulate functional physiology and pathology.[6,7] Furthermore, the intestinal organoid has shown successful models that may simulate the native organ, including gene and protein expression, metabolic activity, tissue engineering, and even pathology, which is greater than 2D cell culture models.[8,9] However, there are obstacles to creating the organoid system, such as constraints in culture system survival and maturity, even cell function, and a high level of cell variability.[9] Furthermore, studies with references showed the

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limitations of survival in the culture system for developing intestinal organoid models.[7,10]

Matrix addition is one of the factors that influences organoid culture outcomes.11 Moreover, matrix addition is a significant factor in developing organoids to decrease cell apoptosis by maintaining survival in the culture system.[12] Culture organoids are grown in matrigel as a matrix.[13,14] However, xenogenic contaminants are found in matrigel, and variability in the composition of matrigel makes the development of organoids challenging.[15,16] Xenogenic contaminants detected in matrigel can interfere with organoid behavior.[16] Furthermore, heterogeneity in the composition of matrigel encourages variability in the physical and biochemical properties of matrigel, which makes it difficult to manipulate cell behavior and obtain precise biological responses.[16] Interestingly, graphene oxide (GO), a synthetic material, has been employed as an artificial matrix in tissue engineering and regeneration, displaying its capacity to direct cell behavior effectively.[17,18] GO can increase mechanical characteristics, promote cell proliferation and adhesion, have a high water absorption capacity, have no effect on cytotoxicity, and even have antioxidant capabilities to neutralize free radicals because GO possesses electrons on its surface.[19] GO has also been demonstrated to regulate gene expression in tissue engineering and regeneration.[20,21] Therefore, GO may have the potential for developing organoid models because it encourages good behavior by creating a microenvironment in vivo.[20,22]

This study aimed to investigate the effects of GO addition as supported factors such as CLS culturing. Maintaining CLS's structures and cell viability in the addition matrix is the first step in developing an intestinal organoid model as a cell-based assay.

## Methods

This study was carried out between February and December 2023 at the Parasitology Laboratory of HSE and FiNder U-CoE Universitas Padjadjaran Bandung. Materials used in this study: phosphate buffered saline/PBS (Sisco Research Laboratories), trypsin/EDTA 0.25% (PAN-Biotech), fetal bovine serum (Sigma-Aldrich), penicillin-streptomycin (PAN-Biotech), gentamicin (PAN-Biotech), Dulbecco's modified eagle medium/DMEM (PAN-Biotech), insulin human recombinant (PAN-Biotech), hydrochloric acid fuming 37% (Supelco), ethanol, graphite (Merck), HCl (Supelco), $NaNO_3$ (Merck), $KMnO_4$, $H_2SO_4$, $H_2O_2$ (Merck), distilled water, silicon oil, ice, NaCl, propidium iodide (Sigma-Aldrich).

GO synthesis is carried out by a modified method based on protocol with references.[23] Graphite (4 g) and 2 g $NaNO_3$ were put into the tube and stirred until mixing. After that, pour $H_2SO_4$ slowly. In the ice bath phase, the tube was placed in a container filled with ice, and the mixture was stirred for 30 minutes. 10 g $KMnO_4$ was added slowly and stirred for 30 minutes. The ice is discarded in the container while the tube remains above the stirrer. The container was filled with silicone oil, and the tube was placed in the container. Increase the temperature by 35–40°C by maintaining the temperatures at 35°C and stirring for 1 hour (at this point, the color typically changes to brown). Then, 90 ml of distilled water was slowly added, and the temperature was raised to 98°C and stirred for 40 minutes (at this point, it usually turned a brownish color that became increasingly clear). Distilled water (400 ml) was added to the mixture, and then 50 ml of $H_2O_2$ was slowly added and stirred for 30 min. Subsequently, the mixture was left overnight.

The following day, after it settles, the clear liquid part is discarded while the settled part is retained. Then, 10 ml of HCl was added, stirred for one hour, and allowed to settle. After it settles, take a temperature measurement and discard the top part of the clear liquid, maintaining the part that has settled. Then, 2,500 ml of distilled water was added, stirred for 30 min, and left to settle (centrifugation stage and repeated pH measurements). Centrifugation (at 10,000 rpm for 5 minutes) was repeated to separate the settled and transparent liquid. In contrast, temperature measurements were carried out to obtain a neutral pH according to the pH of the distilled water used. After the pH reached neutral, it was dried at 60°C.

Structures were characterized using FT-IR (Thermo Scientific Nicolet iS5 in the wavenumber range of 400–4,000 $cm^{-1}$) and particle measurement using particle size analysis/PSA (Horiba Scientific SZ-100).

Ethical approval was obtained from the Research Ethics Committee at the University of

Padjadjaran, 576/UN6.KEP/EC/2023. Male mice of Wistar swabs of 6–12 weeks-old swabs were obtained from the Animal Laboratories Eijkman, Faculty of Medicine, Universitas Padjadjaran. In the present study, the mice were sacrificed by the physical cervical dislocation method.

The CLS–isolation method is modified based on protocol with references.[24,25] A mouse was sacrificed according to ethical approval regulations, dissected, and harvested 15 cm of the small intestine proximal to the stomach. The segment was then placed into a Falcon tube (50 ml) with 15 ml cold PBS (2–8°C) and gentamicin 0.5 mg/ml. Shake the Falcon tube in the circle for 5 minutes to remove contaminants attached to the intestinal segment. Next, tweezers transfer the intestinal segment to a dish containing 10 ml of cold PBS (2–8°C). The intestinal segment was cut lengthwise using scissors and rinsed to remove fat attached to the tissue. Transfer the intestinal segment to a new dish containing cold PBS (2–8°C) and rinse (repeat steps two times, ensure the fatty tissue and contaminants are removed). Cut the intestinal segment into 4–8 mm pieces and place into 50 ml of the Falcon tube with 15 ml cold PBS containing gentamicin (0.5 mg/ml) and vortex at 250 rpm for 5 minutes to remove contaminants. Discard the supernatant, add 15 ml of cold PBS (2–8°C) to a 50 ml Falcon tube, and shake 15–20 times in a circular motion (repeat these steps three times).

After that, add 15 ml of cold PBS (2–8°C) to a 50 ml Falcon tube and use a serological pipette to pipette the intestinal pieces up and down five times gently. After that, shake it 15–20 times in a circular motion, then remove the supernatant (repeat this step 15–20 times or until the supernatant is clear). When the supernatant is clear, remove it and resuspend the tissue pieces in 10 ml of cell dissociation reagent at room temperature (15–25°C) and incubate it for 10 minutes while shaking slowly in a circular motion and allowing the tissue pieces to settle by gravity. Pipette off and discard the supernatant, leaving just enough liquid to cover the tissue pieces. Resuspend the tissue pieces in 10 ml cold PBS (2–8°C) containing 0.1% FBS (2–8°C) and vortex at 250 rpm for 20 seconds, allowing the tissue pieces to settle in the below. Transfer the supernatant carefully with a pipette and filter it through a 70 µm filter.

Then, collect the filtrate in a new 50 ml Falcon tube. Discard the filter, label the filtrate "Fraction 1," and place the fraction on ice. Following that, resuspend the tissue pieces in 10 mL of cold PBS (2–8°C) containing 0.1% FBS (2–8°C) and vortex at 250 rpm for 20 seconds, allowing the tissue pieces to settle in the below (repeat these steps to get Fraction 2–4). After getting four fractions, centrifuge each fraction at 290 RCF for 5 minutes. Pipette off and discard the supernatant, retaining the pellet in each tube. Resuspend each pellet in 10 ml of cold PBS (20–8°C) containing 0.1% FBS (2–8°C), then centrifuge at 200 RCF for 3 minutes. Carefully discard the supernatant, retaining the pellet in each tube.

After obtaining four fractions, resuspend each fraction in 4 ml of cold DMEM/F12 (2–8°C) to prevent and reduce cell damage. Add 1 ml of each fraction using an inverted microscope. Select two fractions enriched with intestinal CLS (CLS that are desirable for culture can be of various sizes, typically rectangular or singular in shape; usually, fractions 3 and 4 are enriched CLS). Mix fractions 3 and 4 and transfer 1 ml to the labeled two Falcon tubes of 15 ml (the number of Falcon tubes is adjusted to the number of treatments; CLS without GO and CLS with GO, then centrifuge each Falcon tube at 200 RCF for 5 minutes and discard the supernatant, retaining the pellet in the bottom of each tube. Next, add 150 µl of the CLS medium at room temperature (15–25°C) to each tube, then add 150 µl of treatment as a matrix (GO) to the labeled Falcon tube. Carefully pipette 50 µl of each suspension into the center of each of the six wells of the pre-warmed 24-well plate. Place the plate at 37°C for 10 minutes, then add 750 µl of medium crypt complete at room temperature (15–25°C) by carefully pipetting the medium down the side wall of each well. Transfer and place the lid on the culture plate, then incubate at 37°C and 5% CO2. Finally, it was observed using an inverted microscope regularly and changed with 750 µl of fresh medium complete at room temperature (15–25°C) three times for a week.

RNA isolation was prepared by harvesting the CLS from each well of the plate with 150 µl cell dissociation reagent in each well of the plate. Then, they are transferred to the labeled Falcon tube of 15 ml containing 1 ml DMEM (the labeled Falcon tube is adjusted for each treatment). Centrifuge at 290 RCF for 3 minutes, discard the supernatant, and retain the pellet. Add 150

**Table 1 Quantitative RT-PCR Primers**

| Primer | Gene for Mouse | Forward Primer | Reverse Primer |
|---|---|---|---|
| Lgr5 | Stem cells intestinal | GACGCTGGGTTATTTCAAGT TCAA | CAGCCAGCTACCAAATAGGT GCTC |
| Vil-1 | Enterocytes | GACGTTTTCACTGCCAATACCA | CCCAAGGCCCTAGTGAAGTCTT |
| GAPDH | Housekeeping gene | AACTTTGGCATTGTG GAAGG | ACACATTGGGGGTAG GAACA |

µl of DMEM to each pellet and then transfer to a microtube. After obtaining CLS, RNA isolation from CLS is received by an RNA extraction kit (MagEX). Besides that, real-time PCR preparation uses mixing PCR (SensiFAST SYBR No-ROX One-Step Kit) and mixing each reagent according to kit composition to each labeled primer microtube. Quantitative RT-PCR was performed with a LightCycler®480 using SYBR Green I/HRM dye (465–510). The sequences of the PCR primer are listed in Table 1.[26] The experiments were carried out in triplicate.

Morphology CLS analysis was examined using a microscope (Inverted Biological Microscope, Labtron Equipment Ltd, UK). The gene expression of CLS measurement was carried out to identify cells of the epithelium intestinal.[26] Propidium iodide (PI) staining to show cell viability of CLS through CLS was seeded onto a 96-well plate and incubated for four days. To investigate the viability of CLS for four days, CLS without and with GO were treated with and without 5FU for four days and stained with PI. 520

Statistical significance was performed using Prism 9 (Grapadh Software, La Jolla, CA, USA). The number of CLS, death cells, and PCR data was analyzed with a t-test. Each experiment was repeated three times. Statistical significance was assumed for p-values<0.05.

## Results

This study shows GO characterization, such as FT-IR and PSA. The varying presence of oxygen functional groups is measured with each wave peak consisting of 3,499 for the O-H group; 1,712 for the C=O group; 1,613 for the C=C group; 1,370 for the C-OH group; and 1,041 for the C-O group as shown in Figure 1A. Besides that, the PSA characterization of GO shows that GO is smaller after 30 minutes of sonication than before sonication, as shown in Figure 1B. The average size distribution of GO after sonication shows 84.5 nm (Figure 1B 2) compared to GO before sonication (Figure 1B 1), namely 697.8 nm. These results indicate that GO is dispersed by the

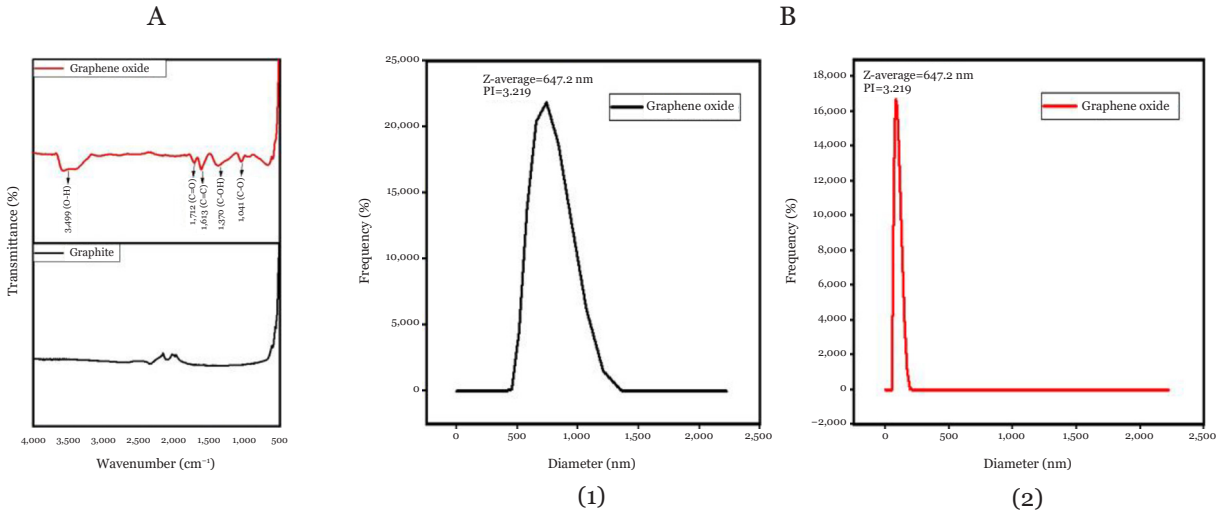


**Figure 1 Graphene Oxide Characterization**
Note: (A) FT-IR for graphite and GO; (B) the PSA analysis of GO before (1) and after (2) sonication

sonication waves, resulting in a smaller particle size.

The CLS isolation has been conducted successfully with enriched CLS as multicellular structures, as indicated by the circular mark for each CLS, as shown in Figure 2A. However, based on this observation, CLS is more enriched in fraction three than in fraction 4. Furthermore, fractions are conducted as CLS, identifying gene expression in the intestinal epithelium. Figure 2E shows the expression of Vil-1 is 1.1 fold and 0.14 fold for CLS primary and intestinal tissue, respectively. Furthermore, the expression Vil-1 in CLS primary was significantly higher than in intestinal tissue (p=0.0333; see Table 2).

Moreover, we seeded CLS without and with GO for five days, as shown in Figure 2B. Based on these results, GO addition can maintain CLS better than without GO addition during incubation time. Furthermore, Figure 2C shows the average number of CLS is 0 and 2.7 for CLS without GO and CLS with GO, respectively. However, the number of CLS without GO is 0 because it is contaminated by yeast (Figure 2B). Besides that, to investigate the effect of GO on maintaining CLS, we seeded CLS without and with GO for seven days (Figure 2C). Figure 2E shows the average number of CLS, which is 3.5 and 14 for CLS without GO and CLS with GO, respectively. In addition, there were no significant differences between the CLS without GO and those with GO (see Table 2). However, CLS GO addition can be maintained as the number of CLS with GO is higher than without GO, as shown in Figure 2E.

Fluorescence staining analyses were performed to investigate whether the viability of CLS can be maintained, which was triggered by 5FU-induced senescence. Figure 3A shows that the group with 5FU treatment demonstrated an alteration in their morphology that became larger and appeared like a dark spot, considered senescence-associated secretory (SASP). In addition, based on this observation, the viability of CLS can be maintained for 96 hours, even after it is treated with 5FU (Figure 3B). Furthermore, Figure 3C shows the average number of cell death is 93 for CLS without 5FU and 230.5 for CLS with 5FU. Based on these results, there were no significant differences among them (see Table 2). However, CLS can be maintained as the number of CLS without 5FU is lower than CLS with 5FU, as shown in Figure 3C.

## Discussion

This study showed that GO has been synthesized successfully. The results of this FT-IR analysis indicate the presence of oxygen functional groups, such as hydroxyl (O-H), epoxy (C-O), carboxyl (C-OH), and carbonyl (C=O) of GO structures.[27] This result supported previous studies.[28,29]

**Table 2 Statistical Analysis with the t-test**

| **t-test Unpaired: Expression of Vil-1** | **Sig.** | **t** | **df** | **F** | **Mean** | **95% CI** |
|---|---|---|---|---|---|---|
| Intestinal tissue | 0.0333* | 3.187 | 4 | 37.05 | 0.1449 | 0.1234 to 1.790 |
| CLS primary | | | | | 1.102 | |
| Difference between ±SEM | | | | | 0.9569±0.3002 | |
| **t-test Paired** | **Sig.** | **t** | **df** | **SD of Difference** | **Mean of Difference** | **95% CI** |
| CLS with and without GO for five days | 0.1567 | 2.219 | 2 | 2.082 | 2.667 | −2.504 to 7.838 |
| CLS with and without GO for seven days | 0.0903 | 7.000 | 1 | 2.121 | 10.50 | −8.559 to 29.56 |
| CLS+GO with and without 5FU for cell viability | 0.2754 | 2.165 | 1 | 89.90 | 137.5 | −669.3 to 944.3 |

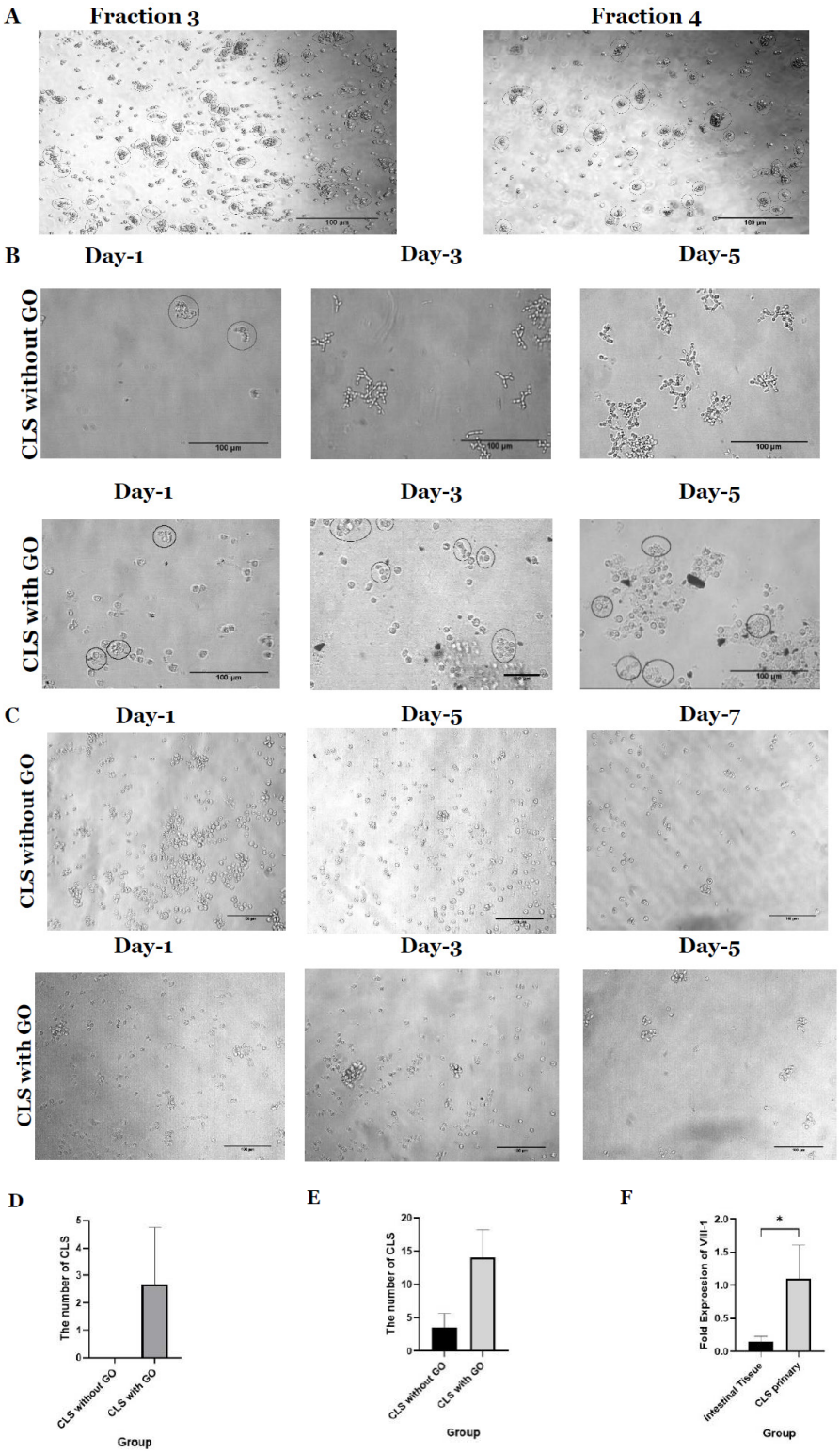


**Figure 2 Crypt-like Structures Characterization**

Note: (A) raw CLS were isolated, scale bar 100 µm with 10× magnification from the small intestines of Wistar swiped mice at 6–12 weeks; (B) CLS is seeded without and with GO for 5 days with 40× magnification, scale bar 100 µm; (C) CLS is seeded without and with GO for 7 days with 20× magnification, scale bar 100 µm; (D) the number of CLS without and with GO was quantified for 5 days; (E) the number of CLS without and with GO was quantified for 7 days; and (F) fold expression of Vil-1 as identified cells in epithelium intestinal with statistical significance considered *p<0.05 (n=2, t-test)

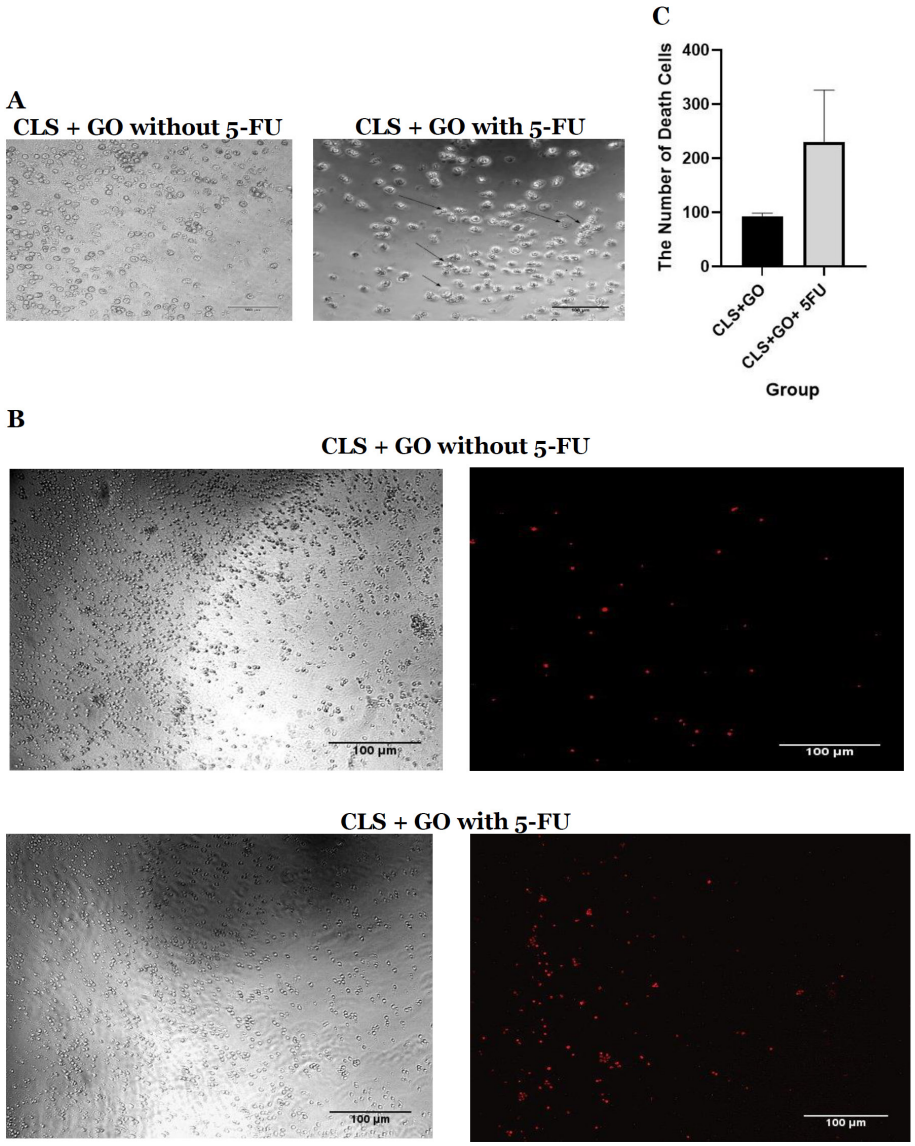


**Figure 3 Cell Viability of CLS**

Note: (A) morphology of CLS+GO without and with 5FU treatment with 20× magnification, scale bar 100 µm; (B) CLS+GO without and with 5FU treatment, before and after PI staining, respectively, with 10× magnification, scale bar 100 µm; and (C) the quantified number of death cells for 4 days

Furthermore, GO, which has been synthesized, was used in this study as a matrix addition for seed CLS isolation. In the present study, CLS isolation morphology has characteristics similar to previous studies.[30,31] These results indicated that CLS isolates have been successfully obtained. Besides that, we received 24 CLS (data not shown). Every crypt contains around 16 cells and around 20–25 cells in the lower and upper columns of the crypt, respectively. Furthermore, the crypt contains around 4–16 stem cells and 4–6 transit-amplifying cells (TA).[32] Crypt obtained from intestinal primary mice can be used for the development of organoids as organ miniatures that represent physiology and pathophysiology response.[33]

Various types of cells differentiate in the intestinal epithelium, such as enterocytes, goblet cells, path cells, and enteroendocrine cells originating from the Lgr5 cell stem found at the base of the intestinal crypt.[34] In this study, CLS isolates are examined for gene expression with genes of interest, such as Lgr5 and Vil-1, as a gene code in stem cells and enterocytes, respectively,

besides Gapdh being used as a housekeeping gene.[26] The housekeeping gene is a gene that is stably expressed in all cells of an organism, regardless of tissue type, developmental stage, and cell cycle state.[35] Gapdh is an internal gene commonly used in studies with gene expression.[36] Previous studies showed that Gapdh is a stable gene used as an internal control in studies using various cells and even for organoid model applications.[37,38]

The present study showed that CSL isolation is difficult to characterize for Lgr5. These results, supported by previous studies, showed that isolating and characterizing primary Lgr5 cells makes obtaining vibrant cultures in epithelial stem cells difficult.[39] It could be because the number of stem cells in the crypt is limited. Each of the crypts comprises approximately 15 stem cells in the mouse.[40] Furthermore, all stem cells compete for niche space between Paneth cells, which provide essential Notch ligands that can only be induced via direct cell-cell contact, so the present cell in a high-WNT environment is the limiting resource in the stem cell zone.[40,41] However, Lgr5 is one of the best characterized of these markers.[42]

Besides that, Vil-1 is used to identify cells in the intestinal epithelium. Vil-1 is a marker that codes enterocytes, the most common cells of the intestinal epithelium, even though it is found along the crypt-villus axis.[40] Furthermore, the small intestine can be identified by the presence of villi, whereas villi don't exist in the caecum and colon.[43] This study showed that fold gene expression of Vil-1 for CLS primary is higher than in intestinal tissue. These results supported the idea that Vil-1 as a marker is used to identify types of cells in the intestinal epithelium.

Besides that, CLS is seeded with GO, which has the highest average number of CLS without GO. These results suggest GO optimization may influence the maintenance of cell complex structures. These results supported that GO addition has effects on cell growth.[18] Besides that, GO-enhanced cell differentiation is required to generate multicellular cells.[17,21,44] Moreover, study with references showed that GO promoted good cell behavior, such as mimicking cell characteristics appropriated to the native cells,[17,20–22] improving proliferation,[18,22] and even regulating gene expression.[18,20–22] Furthermore, due to GO's morphology, shape, size, and even functional groups, it has unique structures and even improves cell interactions.[45] Therefore, in this study, we used the size of GO to be 84.5 nm. Moreover, GO with particle sizes below 100 nm shows no cytotoxic effects.[46]

In addition, CLS is seeded without GO, which is contaminated after the second day of incubation. It may be caused by the lack of control over the environment when the mice were sacrificed. Interestingly, CLS seeded in GO addition demonstrated no contamination. GO has unique physics characteristics, so it is antibacterial.[22] GO has oxygen groups that play significant roles for inactivated bacteria in cells through ROS activation, which induces oxidative stress and encourages an apoptotic pathway.[47] Moreover, a study with references showed that GO has antimicrobial effects, such as antibacterial, anti-fungal, and anti-yeast.48 GO has unique structures, so it has hydrophilic properties that are preferred in cells because it prevents cell agglomeration, impacts nutrient limitation, and even induces oxidative stress, which promotes cell apoptosis.[49] Therefore, GO optimization can maintain the cell viability of CLS. Furthermore, this study showed that the number of death cells in CLS+GO without 5FU was lower than in CLS+GO with 5FU for four days. It indicated that the cell viability of CLS can be maintained.

## Conclusions

In the present study, we found that Vil-1, which is identified as a cell in the intestinal epithelium, is expressed in CLS primary successfully. CLS is seeded with GO addition, which can help maintain multicellular structures. Furthermore, the viability of CLS has shown that it can be maintained. To conclude, this finding supports cell-based assays, as cell viability assays have the potential for developing intestinal organoid models.

## Conflict of Interest

The authors declare no conflict of interest.

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## References


1. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol. 2014;12(4):207–18.
2. Huang SM, Strong JM, Zhang L, Reynolds KS, Nallani S, Temple R, et al. New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. J Clin Pharmacol. 2008;48(6):662–70.
3. Duval K, Grover H, Han LH, Mou Y, Pegoraro AF, Fredberg J, et al. Modeling physiological events in 2D vs. 3D cell culture. Physiology (Bethesda). 2017;32(4):266–77.
4. Fowler S, Chen WLK, Duignan DB, Gupta A, Hariparsad N, Kenny JR, et al. Microphysiological systems for ADME-related applications: current status and recommendations for system development and characterization. Lab Chip. 2020;20(3): 446–67.
5. Baudy AR, Otieno MA, Hewitt P, Gan J, Roth A, Keller D, et al. Liver microphysiological systems development guidelines for safety risk assessment in the pharmaceutical industry. Lab Chip. 2020;20(2):215–25.
6. Danielson JJ, Perez N, Romano JD, Coppens I. Modelling *Toxoplasma gondii* infection in a 3D cell culture system in vitro: comparison with infection in 2D cell monolayers. PLoS One. 2018;13(12):e0208558.
7. Derricott H, Luu L, Fong WY, Hartley CS, Johnston LJ, Armstrong SD, et al. Developing a 3D intestinal epithelium model for livestock species. Cell Tissue Res. 2019;375:409–24.
8. Teriyapirom I, Batista-Rocha AS, Koo BK. Genetic engineering in organoids. J Mol Med (Berl). 2021;99(4):555–68.
9. Hofer M, Lutolf MP. Engineering organoids. Nat Rev Mater. 2021;6(5):402–20.
10. Luu L, Johnston LJ, Derricott H, Armstrong SD, Randle N, Hartley CS, et al. An open-format enteroid culture system for interrogation of interactions between *Toxoplasma gondii* and the intestinal epithelium. Front Cell Infect Microbiol. 2019;9:300.
11. Rauth S, Karmakar S, Batra SK, Ponnusamy MP. Recent advances in organoid development and applications in disease modeling. Biochim Biophys Acta Rev Cancer. 2021;1875(2):188527.
12. El-Badri N, Elkhenany H. Toward the nanoengineering of mature, well-patterned and vascularized organoids. Nanomedicine (Lond). 2021;16(15):1255–8.
13. Huang J, Hume AJ, Abo KM, Werder RB, Villacorta-Martin C, Alysandratos KD, et al. SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. Cell Stem Cell. 2020;27(6):962–73.e7.
14. Engevik MA, Luck B, Visuthranukul C, Ihekweazu FD, Engevik AC, Shi Z, et al. Human-derived *Bifidobacterium dentium* modulates the mammalian serotonergic system and gut-brain axis. Cell Mol Gastroenterol Hepatol. 2021;11(1):221–48.
15. Wang M, Yu H, Zhang T, Cao L, Du Y, Xie Y, et al. In-depth comparison of matrigel dissolving methods on proteomic profiling of organoids. Mol Cell Proteomics. 2022;21(1):100181.
16. Aisenbrey EA, Murphy WL. Synthetic alternatives to matrigel. Nat Rev Mater. 2020;5(7):539–51.
17. Marapureddy SG, Hivare P, Sharma A, Chakraborty J, Ghosh S, Gupta S,, et al. Rheology and direct write printing of chitosan - graphene oxide nanocomposite hydrogels for differentiation of neuroblastoma cells. Carbohydr Polym. 2021;269:118254.
18. Liu W, Luo H, Wei Q, Liu J, Wu J, Zhang Y, et al. Electrochemically derived nanographene oxide activates endothelial tip cells and promotes angiogenesis by binding endogenous lysophosphatidic acid. Bioact Mater. 2021;9:92–104.
19. Abdelhalim AOE, Meshcheriakov AA, Maistrenko DN, Molchanov OE, Ageev SV, Ivanova DA, et al. Graphene oxide enriched with oxygen-containing groups: on the way to an increase of antioxidant activity and biocompatibility. Colloids Surf B Biointerfaces. 2022;210:112232.
20. Zhang J, Yan L, Wei P, Zhou R, Hua C, Xiao M, et al. PEG-GO@XN nanocomposite suppresses breast cancer metastasis via inhibition of mitochondrial oxidative phosphorylation and blockade of epithelial-to-mesenchymal transition. Eur J Pharmacol.

2021;895:173866.
21. Bayaraa O, Dashnyam K, Singh RK, Mandakhbayar N, Lee JH, Park JT, et al. Nanoceria-GO-intercalated multicellular spheroids revascularize and salvage critical ischemic limbs through anti-apoptotic and pro-angiogenic functions. Biomaterials. 2023;292:121914.
22. Zhou D, Liu H, Han L, Liu D, Liu X, Yan Q, et al. Paintable graphene oxide-hybridized soy protein-based biogels for skin radioprotection. Chem Eng J. 2023;469:143914.
23. Bahtiar A, Hardiati MS, Faizal F, Muthukannan V, Panatarani C, Joni IM. Superhydrophobic Ni-reduced graphene oxide hybrid coatings with quasi-periodic spike structures. Nanomaterials. 2022;12(3):314.
24. Chen Y, Li C, Tsai YH, Tseng SH. Intestinal crypt organoid: isolation of intestinal stem cells, in vitro culture, and optical observation. Methods Mol Biol. 2019;1576:215–28.
25. O'Rourke KP, Ackerman S, Dow LE, Lowe SW. Isolation, culture, and maintenance of mouse intestinal stem cells. Bio Protoc. 2016;6(4):e1733.
26. BE, Lee BJ, Lee KJ, Lee M, Lim YJ, Choi JK, et al. A simple and efficient cryopreservation method for mouse small intestinal and colon organoids for regenerative medicine. Biochem Biophys Res Commun. 2022;595:14–21.
27. Bera M, Chandravati, Gupta P, Maji PK. Facile one-pot synthesis of graphene oxide by sonication assisted mechanochemical approach and its surface chemistry. J Nanosci Nanotechnol. 2018;18(2):902–12.
28. Pérez-Molina Á, Morales-Torres S, Maldonado-Hódar FJ, Pastrana-Martínez LM. Functionalized graphene derivatives and TiO2 for high visible light photodegradation of azo dyes. Nanomaterials (Basel). 2020; 10(6):1106.
29. Prodan D, Moldovan M, Furtos G, Saroşi C, Filip M, Perhaiţa I, et al. Synthesis and characterization of some graphene oxide powders used as additives in hydraulic mortars. Appl Sci. 2021;11(23):11330.
30. Wang N, Zhang H, Zhang BQ, Liu W, Zhang Z, Qiao M, et al. Adenovirus-mediated efficient gene transfer into cultured three-dimensional organoids. PLoS One. 2014;9(4):e93608.
31. Han SH, Shim S, Kim MJ, Shin HY, Jang WS, Lee SJ, et al. Long-term culture-induced phenotypic difference and efficient cryopreservation of small Intestinal organoids by treatment timing of Rho kinase inhibitor. World J Gastroenterol. 2017;23(6):964–75.
32. Sumigray KD, Terwilliger M, Lechler T. Morphogenesis and compartmentalization of the intestinal crypt. Dev Cell. 2018;45(2):183–97.e5.
33. Rahmawati L, Puspitasari IM. Teknik pembuatan kultur sel primer, immortal cell line dan stem cell. Farmaka. 2016;14(2):195–206.
34. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. Nature. 2007;449:1003–7.
35. Joshi CJ, Ke W, Drangowska-Way A, O'Rourke EJ, Lewis NE. What are housekeeping genes? PLoS Comput Biol. 2022;18(7):e1010295.
36. Rácz GA, Nagy N, Tóvári J, Apáti Á, Vértessy BG. Identification of new reference genes with stable expression patterns for gene expression studies using human cancer and normal cell lines. Sci Rep. 2021;11(1):19459.
37. Cherubini A, Rusconi F, Lazzari L. Identification of the best housekeeping gene for RT-qPCR analysis of human pancreatic organoids. PLoS One. 2021;16(12):e0260902.
38. Dieterich W, Neurath MF, Zopf Y. Intestinal ex vivo organoid culture reveals altered programmed crypt stem cells in patients with celiac disease. Nat Res. 2020;10(1):3535.
39. Wang X, Yamamoto Y, Wilson LH, Zhang T, Howitt BE, Farrow MA, et al. Cloning and variation of ground state intestinal stem cells. Nature. 2015;522(7555):173–8.
40. Bonis V, Rossell C, Gehart H. The intestinal epithelium – fluid fate and rigid structure from crypt bottom to villus tip. Front Cell Dev Biol. 2021;9:661931.
41. Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell. 2010;143(1):134–44.
42. Dame MK, Attili D, McClintock SD, Dedhia PH, Ouillette P, Hardt O, et al. Identification, isolation and characterization of human Lgr5-positive colon adenoma cells. Development.

2018;145(6):dev153049.
43. Pracht K, Wittner J, Kagerer F, Jäck HM, Schuh W. The intestine: a highly dynamic microenvironment for IgA plasma cells. Front Immunol. 2023;14:1114348.
44. Yao X, Zhan L, Yan Z, Li J, Kong L, Wang X, et al. Non-electric bioelectrical analog strategy by a biophysical-driven nano-micro spatial anisotropic scaffold for regulating stem cell niche and tissue regeneration in a neuronal therapy. Bioact Mater. 2023;20:319–38.
45. Biru EI, Necolau MI, Zainea A, Iovu H. Graphene oxide-protein-based scaffolds for tissue engineering: recent advances and applications. Polymers (Basel). 2022;14(5):1032.
46. Lu P, Zehtab Yazdi A, Han XX, Al Husaini K, Haime J, Waye N, et al. Mechanistic insights into the cytotoxicity of graphene oxide derivatives in mammalian cells. Chem Res Toxicol. 2020;33(9):2247–60.
47. Zhao H, Gu B, Yang P, Yi J, Lv X. Antibacterial properties and mechanism of graphene oxide with different C/O ratio. J Phys Conf Ser. 2023;2468:012002.
48. Asadi Shahi S, Roudbar Mohammadi S, Roudbary M, Delavari H. A new formulation of graphene oxide/fluconazole compound as a promising agent against *Candida albicans*. Prog Biomater. 2019;8(1):43–50.
49. Sekuła-Stryjewska M, Noga S, Dźwigońska M, Adamczyk E, Karnas E, Jagiełło J, et al. Graphene-based materials enhance cardiomyogenic and angiogenic differentiation capacity of human mesenchymal stem cells in vitro – focus on cardiac tissue regeneration. Mater Sci Eng C Mater Biol Appl. 2021;119:111614.

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**RESEARCH ARTICLE**

# Polymorphisms of rs7055763 and rs41307258 in *TBX22* Gene Haplotype as Risk Factors for Non-syndromic Cleft Palate Indonesian Deutero-Malay Population

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**Abstract**

Non-syndromic cleft palate (NS-CP) is a multifactorial congenital malformation affected by genetic and environmental factors. The incidence of non-syndromic cleft lip with or without cleft palate (NS-CLP) varies considerably between ethnic groups and geographical regions. *TBX22* is a crucial determinant for the formation of intramembranous bone in the posterior hard palate. Therefore, *TBX22* is fundamental to palatogenesis and supports normal palate progress. The rs7055763 and rs41307258 polymorphisms in the *TBX22* gene are associated with risk factors for NS-CP in the Indonesian Deutero-Malay population. In the previous study, NS-CP still needed to be investigated in the Deutero-Malay population. However, there are different races, mainly for the Deutero-Malay population. This study aims to determine whether rs7055763 and rs41307258 polymorphisms in the *TBX22* gene are risk factors for NS-CP in the Deutero-Malay population. This study was conducted in Terpadu Laboratory, Faculty of Dentistry, Universitas Padjadjaran, from February until June 2023. The design of this study was a case-control study. The DNA patient samples were obtained from saliva and whole blood. Moreover, DNA is extracted, and the rs7055763 and rs41307258 segments are analyzed using PCR and Sanger sequencing. PCR data was analyzed by chi-square testing. In this study analysis, polymorphisms of rs7055763 (G>A) and rs41307258 (T>A) in the *TBX22* gene show no significant differences between case and control groups, namely 0.911 and 0.645, respectively. However, the genotype in the rs41307258 shows the p-value as 0.027, indicating substantial differences and the OR is 1.390. In conclusion, the rs7055763 and rs41307258 polymorphisms in the *TBX22* gene do not appear to be risk factors for developing NS-CP in the Indonesian Deutero-Malay population.

**Keywords:** Indonesian Deutero-Malay, non-syndromic cleft palate, rs41307258, rs7055763, *TBX22*

## Introduction

Non-syndromic cleft lip with or without cleft palate non-syndromic (NS-CLP) is a congenital disorder also known as orofacial clefting, comprising disorders such as cleft lip (CL), cleft palate (CP) or cleft lip with or without cleft palate (CLP).[1] Furthermore, CLP is a congenital malformation caused by multifactorial factors. It occurs worldwide with a frequency of about 1 in 700 to 1,000 live births, around 45–50% of cases being CL/CP, 25–30% for cleft lip (CL), and 25% for cleft palate (CP).[1–3] Multifactorial factors include genetic and environmental factors that cause non-syndromic CP.[4] Genetic factors, such as physical, chemical, and biological factors, influence the differentiation, migration, and proliferation of neural crest cells, even causing CP. Environmental factors associated with CP include vitamin intake, diet, access to medications, and lifestyle, such as smoking.[5–7] Ethnic and gender differences in the incidence of NS-CLP contribute to a genetic component in the occurrence of the disorder. Moreover, there is a relationship between specific populations and population variations in the incidence of NS-CLP at birth based on geography. The Asian population has the highest prevalence, followed

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by the Caucasian population, and the African population is approximately 1 in 500 births, 1 in 1,000 births, and 1 in 2,500, respectively.[8] In West Java, NS-CLP is around 50.53% for the most significant type of cleft, followed by CL and CP for about 25.5% and 24.42%, respectively.[9]

The prevalence of CP affects about 1 in 1,000 live births worldwide.[10] NS-CP incidence varies considerably between ethnic groups and geographical regions.[11] Furthermore, NS-CP refers to any palate cleft located posterior to the incisive foramen, which does not involve the alveolar processus or the lip.[12] In Indonesia, the birth prevalence of orofacial clefts, including NS-CP, is around 0.2%, with a total of 7,500 cases reported each year. This incident highlights the need for special attention in the research and treatment of this disorder in Indonesia, considering the long-term impact on the quality of life of patients and their families.[13] NS-CP influences sufferers are psychological problems caused by feelings of shame. Besides that, NS-CP disorders can also cause eating disorders in sufferers. Furthermore, especially for babies with NS-CP, parents will have difficulty feeding their baby on the first day of birth, so special feeding aids are needed. Another prominent problem that NS-CP sufferers can experience is speech disorders and nasal sounds.[1,14] Besides that, the Deutero-Malay population is one of the ethnic groups in Indonesia consisting of Malay, Makassar, Javanese, Sundanese, Bugis, and Minang people, even if it has a unique genetic background that may influence the frequency and impact of NS-CP.[15–17] Moreover, the genetic factors that influence NS-CP in the Deutero-Malay Indonesian population are rs2235373 and rs2235371 polymorphisms in the *IRF6* gene showed that the rs2235373 polymorphism influences the functional role of the gene, and it is a risk factor for NS-CP incidence.[17–19] Besides that, the various genes that contributed to the occurrence of NS-CP are very diverse, such as interferon regulatory factor 6 (*IRF6*), homeobox gene 1 (*MSX1*), methylenetetrahydrofolate reductase (*MTHFR*), T-box 22 (*TBX22*).[17,18,20–22] The *TBX22* gene is located on the X chromosome, and it is part of the T-box gene family, which plays a vital role in embryonic development, including craniofacial development in NS-CP. Furthermore, genetic and environmental factors showed that about 50% influence NS-CP risk.[23] The genetic and environmental factors include growth, DNA transcription, nutrient metabolism, immunity, and oncogenesis.[22] The *TBX22* gene, which encodes a T-box transcription factor, is a candidate gene associated with NS-CP incidence. *TBX22* gene consists of nine exons at position Xq21.1 and has two promoters, P0 and P1.[24] Furthermore, the P0 promoter contains the rs7055763 and rs41307258 polymorphisms. Single nucleotide polymorphisms (SNPs) can affect gene function and play a role in disease mechanisms, susceptibility to environmental factors, and the increase or inheritance of the risk of certain diseases. Inherited polymorphisms such as rs7055763 and rs41307258 together are referred to as haplotypes.[25,26]

The various studies showed that the mutations in *TBX22* were consistently found in NS-CP patients in Thai, Brazilian, North American, and Indian populations.[24,27,28] The role of the *TBX22* gene in NS-CP has yet to be investigated in the Indonesian Deutero-Malay population.*16–18* Interestingly, previous studies concluded that the *TBX22* gene is significantly associated with NS-CP incidence.[2,24,27] Therefore, this study aims to determine whether the rs7055763 and rs41307258 polymorphism in the *TBX22* gene are risk factors for NS-CP in the Deutero-Malay population.

## Methods

This study was conducted in Laboratorium Penelitian Terpadu and Foundation for the CL/CP sufferers in Dentistry of Universitas Padjadjaran from February until June 2023. This study used convenience sampling based on inclusion and exclusion criteria. Furthermore, to be eligible to participate in this study, a participant must be NS-CP without any other abnormalities, willing to be included as a patient subject, while healthy individuals with no relatives who have had a cleft palate in the previous two generations and willing to be included as a control subject. Participants with NS-CP who do not belong to the Deutero-Malay community are excluded. The subjects of this study were 29 patients with NS-CP from the Deutero-Malay population who came to the Cleft Lip and Palate Foundation.

Moreover, 58 patients were selected from healthy individuals without NS-CP and had a family history of NS-CP at least two generations

ago as the control subjects. The sample used in this study was blood or saliva. The sample that forms blood was collected by injection technique, while saliva was taken by gargle. This sampling technique is explained further. First, tie the arm to slow blood flow so that the veins are more clearly visible and the blood sample is easy to take. After that, they cleaned the sampling area with tissue or cotton containing alcohol. Then, a syringe is inserted to take the blood sample, followed by a transfer to the EDTA tube. The saliva sample is conducted by the requirement of the respondents in this study not to eat, drink, gargle, brush their teeth, or smoke for 1 hour before taking the saliva; in addition, the respondents can take a breath, following that is hard coughing.

After that, the respondents were asked to gargle with the mouthwash for 10–15 seconds. Then, hold it in the mouth and repeat to gargle 3–5 times. Finally, they collected the fluids in the mouth into a tube and added them to the mixing solution. Shake it till frothy, then wrap it with biohazard plastics. The ethics of this study have been approved by the Research Ethics Committee of Universitas Padjadjaran, number 529/UN6. KEP/EC/2023. Moreover, the materials of this study consist of cell lysis solution containing ten mM Tris-HCL with pH 8.0, 25 mM disodium EDTA, sodium dodecyl sulfate (SDS) 0.5%, K proteinase, RNA-ase, protein precipitation solution (ammonium acetate 5M), absolute isopropanol, cold alcohol (70%), and TE buffer 1X.

The study design is a molecular epidemiology study with an analytical case-control observation. The working method involved DNA isolation from the participant's whole blood and saliva. The first step is to gargle to generate saliva, while an injection procedure is used to collect a whole blood sample. Moreover, the method is based on protocol with references to get the saliva sample and the entire blood.[29–33] First, saliva samples or 300 µl whole blood were placed in a 1.5 ml microtube and centrifuged at around 13,000–16,000 rpm for 30 seconds. Add 900 µl RBC lysis solution for the entire blood samples and incubate for 10 minutes at room temperature. Next, the leucosis pellet was centrifuged at about 13,000–16,000 rpm for 20 seconds, then of the supernatant (repeat this step until the red of the leucosis fades (for the whole blood) and the pellet is thick (for saliva samples)). Subsequently, 300 µl cell lysis solution was added into the microtube containing the cell pellets and homogenized through up and down pipetting. Added 15 µl K proteinase, then homogenize and incubate at 55°C for 30 minutes. After that, add 1,5 µl RNAse, homogenize, and incubate at 37°C for 15 minutes. After adding 100 µl of the protein precipitation solution to the microtube, it was vortexed for 15 seconds and then centrifuged for 3 minutes. Transferred the supernatant to the 1.5 ml new microtube and added 600 µl isopropanol. Next, shake the microtube ten times until the clod is formed. To extract the DNA pellet, centrifuge the microtube for one minute. Following that, the cold 600 µl alcohol (70%) was used to wash the DNA pellet and centrifuged for one minute to remove the alcohol and obtain the DNA pellet. Reverse the tube and let the DNA pellet dry on the tissue until it evaporates. Lastly, 50 µl of TE buffer was added to dissolve the DNA pellet.

In addition, the measurement of DNA fragments in the promoter region of the *TBX22* gene used the polymerase chain reaction (PCR) technique (thermo fisher PCR). Furthermore, the method of this study used primers for the rs7055763 and rs41307258 polymorphisms with lengths of about 125 bp and 556 bp, respectively. The forward and reverse primers of the polymorphisms in the *TBX22* gene were included from the forward primer 5'-3': GAGCTGCCCTGGAGAAATAA and reverse primer 5'-3': AGCACAAGAGAACGTGGTGT. Besides that, electrophoresis was performed to ensure that the PCR products from the amplification had the desired base pair number.

Besides that, sequencing was then carried out to determine a nucleotide. In this study, PT Genetika Science carried out the sequencing technique. Furthermore, a sequencing technique is performed to analyze polymorphisms rs7055763 and rs41307258 in the genetic variation. This begins with the preparation of sequencing reactions using the Sanger dideoxy method to determine the nucleotide sequence. The sequencing results are usually received in files with a .ab1 extension. Several programs must be installed to open and analyze these files. The sequencing results are then completed with chromatograms and nucleotide sequences. Furthermore, chromatograms are represented by the colored lines of the sequence of DNA bases, with adenine bases shown in green, guanine

bases in black, thymine bases in red, and cytosine bases in blue. The polymorphisms of rs7055763 and rs41307258 in patients with non-syndromic cleft palate were observed using sequencing techniques.

Moreover, the sequencing results are formed by chromatogram data displayed by Chromas 2.6.5. BioEdit program is used for nucleotide sequence analysis. Furthermore, it analyzes nucleotide sequences of the case and control case samples and compares them with the reference nucleotide base sequence. Meanwhile, statistical significance was obtained using SPSS statistics 26. The PCR data was analyzed using the chi-square test. Statistical significance was assumed for p-values <0.05.

## Results

The samples were collected from the blood or saliva forms based on the patient and control subjects; then, DNA extraction was carried out for further PCR analysis. After several optimization attempts, an optimal condition led to single-band PCR products. The overall optimal PCR results of the non-syndromic cleft palate case and control samples were obtained, followed by sequencing techniques to analyze the genetic variations at rs7055763 and rs41307258 polymorphisms. In addition, the PCR products generated in this study for the polymorphisms rs7055763 and rs41307258 in *TBX22* genes had a size of 681 bp.

Figure 1 and Figure 2 show the image of the design primer and the location of rs7055763 and rs41307258 in the *TBX22* gene, respectively, and the electrophoresis image of the *TBX22* PCR products.

The variations in the base sequence of the gene, as depicted by the arrow in Figure 3 and Figure 4, have potential implications. The green-colored curve, indicating homozygous genetic variation, and the single dominant A signal, suggesting a homozygous AA base, could have significant implications. Similarly, the black-colored growth curve marked with the letter G (GG) and the genetic variation in heterozygotes indicated by green and black growth curves with two dominant signals for A and G bases (GA) could also have important implications. These findings, represented in R code, demonstrate the genetic variations of homozygous (AA), wild type (GG), and heterozygous (GA) in the rs7055763 polymorphism, as shown in Figure 3.

Figure 4 presents the genetic variations of homozygous (AA), wild-type (TT), and heterozygous (TA) in the rs41307258 polymorphism with utmost clarity. The change from the T allele, the wild-type allele, to the A allele, which denotes the polymorphic allele, is clearly explained. The green-colored curve, indicating


**Figure 1 Design Primer and the Location of rs7055763 and rs41307258 in *TBX22* Gene**

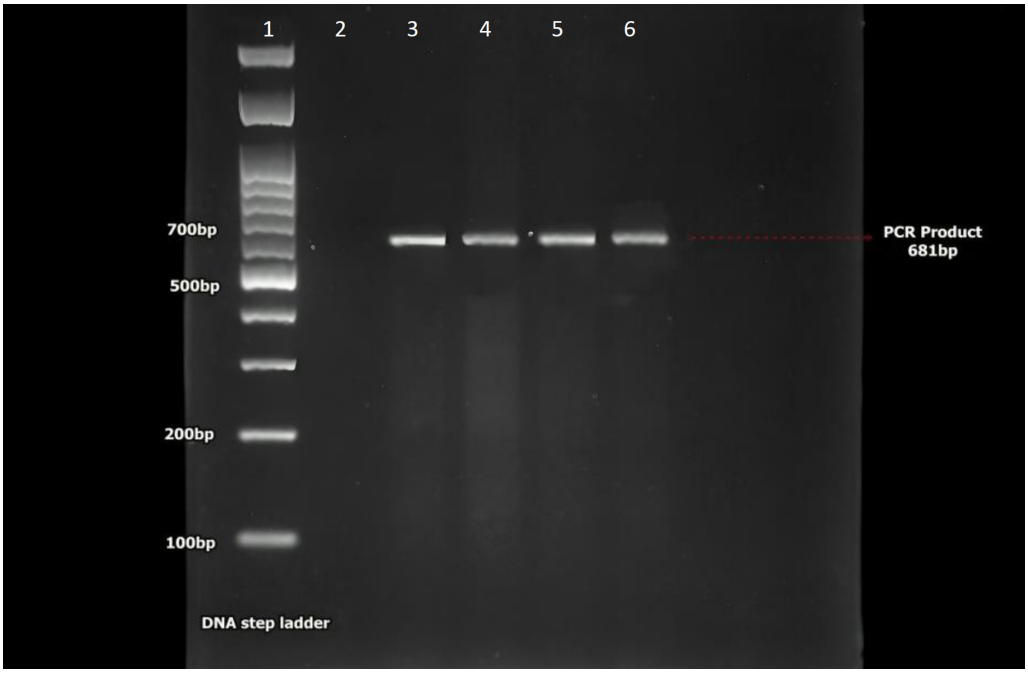


**Figure 2 PCR Products of the *TBX22* Gene for rs7055763 and rs41307258**
Note: for each track in the number (1) DNA ladder, (2) control negative, (3) PCR products with the size in 681 bp

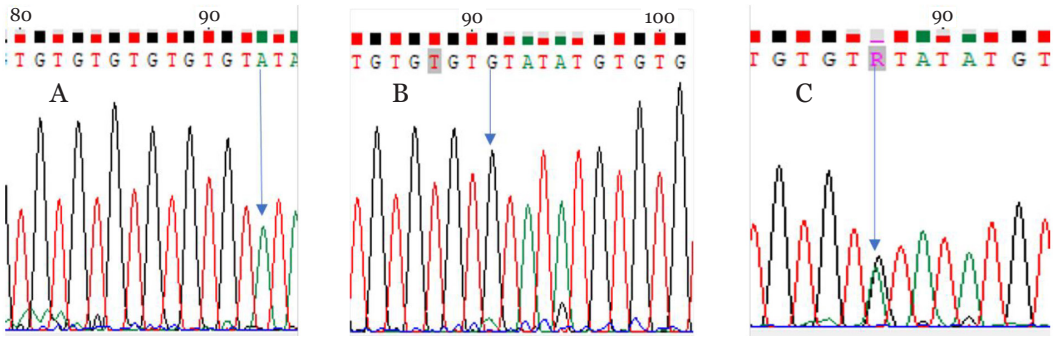


**Figure 3 Chromatogram for rs7055763**
Note: (A) homozygous (indicated by the green curve), (B) homozygous (indicated by the black curve), (C) heterozygous (indicated by both green and black curves)

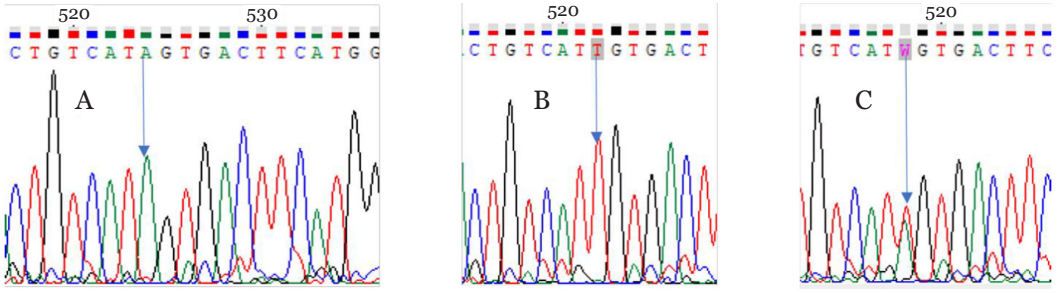


**Figure 4 Chromatogram for rs41307258**
Note: (A) homozygous (indicated by the green curve), (B) is homozygous (indicated by the red curve), (C) heterozygous (indicated by both green and red curves)

homozygous genetic variation, and the single dominant A signal, suggesting a homozygous AA base, are unmistakable. Similarly, the red-colored growth curve marked with the letter T (TT) and the heterozygous genetic variation indicated by green and red growth curves with two dominant signals for A- and T-bases are presented in a way that leaves no room for doubt. These results are referred to as the A W code, providing a clear understanding of the genetic variations.

Table 1 compares genotypes and alleles of the rs57055763 and rs41307258 polymorphisms in the *TBX22* gene for the case and control group. Our thorough research includes comparing genotypes and alleles of rs57055763 polymorphism in the *TBX22* gene, which showed a p-value of about 0.283 and 0.911, and rs41307258 polymorphism, which showed a p-value of about 0.027 and 0.645. The p-value of comparing haplotypes in the case and control groups of the GT haplotype, GA* haplotype, AT haplotype, and AA* haplotype is 0.984, as summarized in Table 2. The number of cases and controls is 34, 78, and 116 (see Tables 1 and 2), indicating total interaction between each group.

## Discussion

This study focused on the genetic polymorphisms of the rs7055763 and rs41307258 in the *TBX22*

**Table 1 Comparison of Genotype and Allele of the rs7055763 and rs41307258 Polymorphisms in *TBX22* in the NS-CLP Case and Control Groups**

| Genotype/Allele | Groups | | OR (95% CI) | p | $\chi^2$ |
|---|---|---|---|---|---|
| | Case n=29 (%) | Control n=58 (%) | | | |
| Genotype of *TBX22* rs7055763 | | | | 0.283 | 2.527 |
| GG | 16 (52) | 28 (48) | 2.171 (0.680–6.933) | | |
| GA | 5 (17) | 19 (33) | 0.362 (0.095–1.384) | | |
| AA | 8 (28) | 11 (19) | 0.786 (0.262–2.357) | | |
| Allele *TBX22* rs7055763 | n=58 (%) | n=116 (%) | 0.963 (0.499–1.858) | 0.911 | 0.013 |
| G | 37 (64) | 75 (65) | | | |
| A | 21 (36) | 41 (35) | | | |
| Genotype of *TBX22* rs41307258 | n=29 (%) | n=58 (%) | | 0.027* | 7.217 |
| TT | 20 (69) | 31 (53) | 10.323 (1.268–84.046) | | |
| TA | 1 (3) | 16 (28) | 0.086 (0.009–0.788) | | |
| AA | 8 (28) | 11 (19) | 0.887 (0.304–2.587) | | |
| Allele *TBX22* rs41307258 | n=58 (%) | n=116 (%) | 1.175 (0.592–2.332) | 0.645 | 0.213 |
| T | 41 (71) | 78 (67) | | | |
| A | 17 (29) | 38(33) | | | |

**Table 2 Comparison of Haplotypes in the Case and Control Groups**

| Haplotype | Groups | | OR (95% CI) | p | $\chi^2$ |
|---|---|---|---|---|---|
| | Case n=29 (%) | Control n=58 (%) | | | |
| GT | 21 (61.8) | 47 (60.2) | 0 (0.0–0.0) | 0.984 | 0.459 |
| GA* | 0 (0.0) | 1 (1.3) | 0 (0.0–0.0) | | |
| AT | 4 (11.7) | 2 (2.6) | 6.222 (0.972–39.814) | | |
| AA* | 9 (26.5) | 28 (35.9) | 1.390 (0.5599–3.454) | | |

associated with haplotypes as risk factors for NS-CP incidence in the Indonesian Deutero-Malay population. Dixon et al.[8] found that the highest incidence was in Asian populations. Furthermore, the study by Sjamsudin and Maifara[9] showed that the percentage incidence of CP in the population of West Java between 2011 and 2015 was around 25.05%. Besides that, other studies explained that the CP incidence occurs more frequently in male patients than female patients. In addition, the survey by Pauws et al.[24] revealed that the incidence of CP occurs frequently in female patients because the rs7055763 and rs41307258 polymorphisms are located in promotor and associated with the CP incidence. The other study by Gurramkonda et al.[28] showed that rs7055763 and rs41307258 polymorphisms increased the NS-CP incidence in South India, as indicated by AA haplotypes. Besides that, one-fifth of patients have a family history of CP. In addition, most patients with CP have low socioeconomic status, which is considered an environmental factor10, but ecological factors were not considered in this study.

Another study by Burg et al.[34] demonstrated the association of the *TBX22* gene with the incidence of CP. *TBX22*, a gene that plays a crucial role in palatogenesis, is found to be associated with the development of CP. The rs7055763 and rs41307258 polymorphisms in the *TBX22* gene are located in the promoter region, which is upstream of the transcription start site and is involved in the initiation of the transcription process. This study reveals that the nucleotide base polymorphism of rs7055763 changes from G to A, while the polymorphism of rs41307258 changes from T to A. The interaction between the polymorphisms of rs7055763 G/A (G>A) and rs41307258 T/A (T>A) leads to GT, GA, AT, and AA. Errors in the DNA sequence during the transcription process can alter the structure and function of the resulting product, leading to the appearance of different phenotypic traits in the organism. This provides a clearer understanding of the genetic basis of CP.

Eighty-seven subjects, carefully selected based on the inclusion and exclusion criteria, were part of this study. The results, which revealed all samples had an optimal PCR product labeled with single bands, are significant. The sequencing results of the rs7055763 and rs41307258 polymorphisms in the *TBX22* gene, compared with human nucleotide sequence data in the DNA bank, provide crucial genetic variation. This study's findings shed light on the significant impact of genetic factors on NS-CP incidence in the Indonesian Deutero-Malay population, including the *IRF6* polymorphisms of rs2235373 and rs2235371. The discovery that the rs2235373 polymorphism affects the functional role of the gene and the action, making it a risk factor for the incidence of NS-CP, is particularly enlightening. Additionally, the association of *MTHFR* A1298C rs1801131 with NS-CP in the Indonesian Deutero-Malay population is a significant finding.

The study by Suphapeetiporn et al.,[27] which showed that the mutation of *TBX22* frequently causes NS-CP in the Thai population, has significant implications. This study aimed to investigate whether the mutations in *TBX22* play a role in the formation of NS-CP in the Thai population, a question of great interest to the medical community. The mutations in the *TBX22* gene were performed in 53 Thai patients unrelated to NS-CP, further engaging the audience. The discovery that the mutations in the *TBX22* gene are responsible for a significant incidence of NS-CP cases in Thailand is a finding of great interest. The study by Pauws et al.,[24] which showed that the rs41307258 polymorphism plays a crucial functional role in Brazil, America, and European populations, also has significant implications. The result showed that the promoter activity of the *TBX22* gene declines by around 50%, leading to the incidence of CP, a finding that will surely pique the audience's interest. Haplotypes containing the rs41307258 promoter are associated with decreased transcriptional activity of *TBX22*, further engaging the audience.

Another study by Fu et al.[10] showed that loss of function mutations in the X-linked *TBX22* promoter disrupts the ETS-1 binding site and causes NS-CP incidence. Furthermore, the incidence of NS-CP is identified by the ÿ73G>A mutation in the X-linked *TBX22* promoter. Therefore, the X-linked *TBX22* promoter mutations can cause CP by disrupting the *TBX22*-ETS-1 pathway. Furthermore, the X chromosome harbors the genomic region of the *TBX22* gene, which is associated with the vital role of transcription factors in mammalian cell differentiation and embryonic development. Gurramkonda et al.[28] reported that the rs7055763 and rs41307258 polymorphisms in the *TBX22*

gene are located in the P0 promoters developing pathogenesis of NS-CP in the Indian population. Furthermore, the result showed that the rs7055763 and rs41307258 polymorphisms had a significant p-value in women with NS-CP, for around 0.034 and 0.022, respectively. Therefore, the two polymorphisms with heterozygous and homozygous variation increased the risk of developing NS-CP in women. However, the rs7055763 and rs41307258 polymorphisms were not significant in men. In the AA haplotype, carrying both mutant alleles (rs7055763 A - rs41307258 A) was significantly associated with NS-CP risk in women but not men. Moreover, the SNPs were not associated with NS-CP risk in men.

This study investigated the role of the rs7055763 and rs41307258 polymorphisms in the *TBX22* gene related to NS-CP incidence in the Indonesian Deutero-Malay population. Moreover, the rs7055763 polymorphism in the *TBX22* gene shows a positive correlation of allele A and genotype AA with the NS-CP phenotype. Besides that, there were more allele A and genotype AA in the NS-CP group than in the control group. However, this result is not statistically significant. At the same time, the rs41307258 polymorphism in the *TBX22* geneshows a positive correlation of allele T and genotype TT with the NS-CP phenotype. In addition, there were more alleles T and genotype TT in the NS-CP group than in the control group. Therefore, this study showed that rs41307258 and rs7055763 polymorphisms in the *TBX22* gene as a risk factor for NS-CP in genotype, allele, and haplotype in the case and control groups show no statistically significant. However, the limitation of this study is that the sample size may influence these results. Besides that, the genotype for the rs41307258 in the *TBX22* gene is statistically significant, as summarized in Table 2. In addition, the odds ratio shows the value is more than 1, indicating potential polymorphisms as risk factors for NS-CP incidence. However, further study is needed to increase the number of case samples in rs7055763 - rs41307258 polymorphisms of the *TBX22* gene, even compared with other polymorphisms for analyzing their interaction.

## Conclusion

In the Indonesian Deutero-Malay population, the rs7055763 and rs41307258 polymorphisms in the *TBX22* gene are not risk factors for developing non-syndromic cleft palate.

## Conflict of Interest

The authors declared no conflict of interest.

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## References

1. Shehzad H, Shehzad O. Detection of single nucleotide polymorphism rs2013162 of *IRF6* gene in a cleft lip and palate patient. Int J Front Sci. 2018;3(1):28–40.
2. Buana A, Aziz WV. Pola penurunan alel polimorfisme gen *TGFβ3* rs2300607 T>A pada penderita celah bibir dan langit-langit non sindromik (CB/L NS). In: Paryati SPY, Suhartono, Djamal EC, Murniati A, Najmurrokhman A, editors. Prosiding SNIJA 2015. Cimahi: Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) Universitas Jenderal Achmad Yani (Unjani); 2015. p. 45–9.
3. Nasroen SL. Celah bibir dan langit-langit non sindromik: pemahaman dan pendekatan polimorfisme gen. Banda Aceh: Syiah Kuala University Press; 2022.
4. Menet R, Bernard M, ElAli A. Hyperlipidemia in stroke pathobiology and therapy: insights and perspectives. Front Physiol. 2018;9:488.
5. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. Am J Med Genet Part C Semin Med Genet. 2013;163C(4):246–58.
6. Kamiloglu B. Presurgical treatment of cleft lip and palate babies with a PNAM appliance: a series of four case reports. J Interdiscipl Med Dent Sci. 2014;2(6):1000148.
7. Allam E, Windsor LJ, Stone C. Cleft lip and palate: etiology, epidemiology, preventive and intervention strategies. Anat Physiol. 2014;4(3):1000150.
8. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding

genetic and environmental influences. Nat Rev Genet. 2011;12(3):167–78.
9. Sjamsudin E, Maifara D. Epidemiology and characteristics of cleft lip and palate and the influence of consanguinity and socioeconomic in West Java, Indonesia: a five-year retrospective study. Int J Oral Maxillofac Surg. 2017;46(Suppl 1):S69.
10. Fu X, Cheng Y, Yuan J, Huang C, Cheng H, Zhou R. Loss-of-function mutation in the X-linked *TBX22* promoter disrupts an ETS-1 binding site and leads to cleft palate. Hum Genet. 2015;134(2):147–58.
11. Li Q, Xu L, Jia X, Saleem K, Zaib T, Sun W, et al. SNPs in folate pathway are associated with the risk of nonsyndromic cleft lip with or without cleft palate, a meta-analysis. Biosci Rep. 2020;40(3):BSR20194261.
12. Martinelli M, Palmieri A, Carinci F, Scapoli L. Non-syndromic cleft palate: an overview on human genetic and environmental risk factors. Front Cell Dev Biol. 2020;8:592271.
13. Putri FA, Pattamatta M, Anita SES, Maulina T. The global occurrences of cleft lip and palate in pediatric patients and their association with demographic factors: a narrative review. Children (Basel). 2024;11(3):322.
14. de Vries IAC, Breugem CC, van der Heul AMB, Eijkemans MJC, Kon M, Mink van der Molen AB. Prevalence of feeding disorders in children with cleft palate only: a retrospective study. Clin Oral Investig. 2014;18(5):1507–15.
15. Hatin WI, Nur-Shafawati AR, Zahri MK, Xu S, Jin L, Tan SG, et al. Population genetic structure of peninsular Malaysia Malay sub-ethnic groups. PLoS One. 2011;6(4):e18312.
16. Setiawan J, Permatasari WI. Proses masuk dan persebaran peninggalan kebudayaan Proto-Deutero Melayu di Indonesia. Fajar Historia. 2019;3(1):11–22.
17. Maskoen AM, Nasroen SL, Yazid H, Fauziah PN, Soemantri ESS. Sequence variants in Exon 1 of MSX1 gene associated with non-syndromic cleft lip/palate (NS CL/P) among Indonesian patients. Int J Chemtech Res. 2016;9(8):557–63.
18. Nasroen SL, Maskoen AM, Soedjana H, Soemantri SS, Hilmanto D. The effects of *IRF6* rs2235373 polymorphism on mRNA expression changes in non-syndromic cleft lip and palate with various phenotypes. Padjadjaran J Dent. 2018;30(3):222–32.
19. Nasroen SL, Tammama T, Putri GAN. *MTHFR* C677T rs1801133 gene polymorphism as a risk factor for nonsyndromic cleft palate only among Deutero Malay sub race in Indonesia. JHDS. 2022;Spec:195–208.
20. Khan MI, CS P. Case–parent trio studies in cleft lip and palate. Glob Med Genet. 2020;7(03):75–9.
21. Chiquet BT, Henry R, Burt A, Mulliken JB, Stal S, Blanton SH, et al. Nonsyndromic cleft lip and palate: CRISPLD genes and the folate gene pathway connection. Birth Defects Res A Clin Mol Teratol. 2011;91(1):44–9.
22. Levi B, Brugman S, Wong VW, Grova M, Longaker MT, Wan DC. Palatogenesis: engineering, pathways and pathologies. Organogenesis. 2011;7(4):242–54.
23. Ahmed MK, Bui AH, Taioli E. Epidemiology of cleft lip and palate. In: Almasri MA, editor. Designing strategies for cleft lip and palate care [e-book]. London: IntechOpen; 2017 [cited 2024 June 10]: 3–22. Available from: https://www.intechopen.com/chapters/53918.
24. Pauws E, Moore GE, Stanier P. A functional haplotype variant in the *TBX22* promoter is associated with cleft palate and ankyloglossia. J Med Genet. 2009;46(8):555–61.
25. Pauws E, Hoshino A, Bentley L, Prajapati S, Keller C, Hammond P, et al. *Tbx22*$^{null}$ mice have a submucous cleft palate due to reduced palatal bone formation and also display ankyloglossia and choanal atresia phenotypes. Hum Mol Genet. 2010;19(15):3103.
26. Ismail S, Essawi M. Genetic polymorphism studies in humans. Middle East J Med Genet. 2012;1(2):57–63.
27. Suphapeetiporn K, Tongkobpetch S, Siriwan P, Shotelersuk V. *TBX22* mutations are a frequent cause of non-syndromic cleft palate in the Thai population. Clin Genet. 2007;72(5):478–83.
28. Gurramkonda VB, Hussain SA, Murthy J, Lakkakula BVKS. Two promoter polymorphisms in *TBX22* are associated with the risk of NSCLP in Indian women. Clin Dysmorphol. 2015;24(4):140–3.
29. Maksum IP, Sriwidodo, Gaffar S, Hassan K, Subroto T, Soemitro S. Teknik biologi molekular. Sumedang: Alqaprint Jatinangor; 2017.

30. Guha P, Das A, Dutta S, Chaudhuri TK. A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. J Clin Lab Anal. 2018;32(1):e22181.
31. Samadi Shams S, Zununi Vahed S, Soltanzad F, Kafil V, Barzegari A, Atashpaz S, et al. Highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples. Bioimpacts. 2011;1(3):183–7.
32. Garbieri TF, Brozoski DT, Dionísio TJ, Santos CF, Neves LT. Human DNA extraction from whole saliva that was fresh or stored for 3, 6 or 12 months using five different protocols. J Appl Oral Sci. 2017;25(2):147–58.
33. Sigma-Aldrich. Saliva DNA extraction & WGA amplification [Internet]. St. Louis: Sigma-Aldrich; 2024 [cited 2024 June 15]. Available from: https://www.sigmaaldrich.com/ID/en/technical-documents/protocol/genomics/dna-and-rna-purification/extraction-of-dna-from-saliva.
34. Burg ML, Chai Y, Yao CA, Magee W 3rd, Figueiredo JC. Epidemiology, etiology, and treatment of isolated cleft palate. Front Physiol. 2016;7:67.

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**RESEARCH ARTICLE**

# Association between Clinical Characteristics of Pregnant Women in the Third Trimester and Low Back Pain

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**Abstract**

Pregnancy is a physiological condition characterized by growth in both the fetus and the mother. Various biomechanical, physiological, and structural changes in pregnant women cause body posture changes, impacting low back pain (LBP). In the third trimester, LBP pain felt by pregnant women is usually accompanied by activity limitations and a decreased quality of life. This study aimed to determine the association between the clinical characteristics of pregnant women in the third trimester and low back pain. This research was conducted at the Department of Obstetrics and Gynecology and Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung from June to December 2017. The method is observational analysis with a cross-sectional approach with a sample of pregnant women in their third trimester who complained of lower back pain and lived in Bandung. According to the findings, the majority of pregnant women with LBP in the third trimester were under the age of 35 (33 of 38), had multiparous parity (25 of 38), had normal pre-pregnancy weight (21 of 38), gained an average of 11.28 kg during pregnancy, and had a median pain intensity of 5. Age was the only factor significantly correlated with LBP pain intensity; parity and weight gain during pregnancy did not. The study concludes a significant association between age in the third trimester of pregnancy and the intensity of LBP pain. Still, no significant association was found between parity and weight gain during pregnancy with the intensity of LBP pain.

**Keywords:** Low back pain, pain intensity, pregnancy, third trimester

## Introduction

Pregnancy is a physiological condition characterized by growth in both the fetus and the mother. Biomechanical, physiological, and structural changes occur in pregnant women. This situation causes changes in body posture which results in low back pain.[1,2]

Low back pain (LBP) affects the muscles, nerves, and spine below the costal margin and the inferior gluteal folds.[3,4] LBP in pregnancy has become a global issue, affecting both developed and developing countries.[5] The prevalence of LBP during pregnancy differs per subregion, ranging from 24 to 90%.[6] Clinical characteristics of patients, such as age and parity, have been indicated as the most common risk factors for LBP.[7] The mother's pre-pregnancy weight and BMI are also risk factors for LBP during pregnancy. LBP usually begins to appear in pregnant women in the second and third trimesters of pregnancy.[8]

The peak severity of LBP occurs in the third trimester. Previous studies have shown that the prevalence of LBP increases with gestational age, reaching 20% before pregnancy, 40% in the first trimester, and 44–70% in the third trimester.[9] As the gestational age increases, there is an increase in the lumbar lordotic curve, causing LBP and limited movement, which ultimately leads to activity restrictions.[10] During the third trimester, pregnant women's pain is frequently accompanied by a decrease in activity and quality of life.[11] LBP frequently interferes with activities and negatively impacts the life quality of pregnant women. Previous research indicates that one-third of the population suffering from LBP reports that acute pain is frequently related to limitations in women's capacity to work efficiently, resulting in a low quality of life.[12] As a result, women's productivity in routine daily activities is reduced.

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Understanding the association between clinical characteristics of pregnant women and LBP, as defined by pain intensity indicators, is crucial for improving the quality of health care, particularly for pregnant women in the third trimester. Previous studies have discussed the association between LBP and the clinical characteristics of pregnant women, but not many have focused on the association among pregnant women in the third trimester.[13,14] This research aims to fill this gap by determining the association between the clinical characteristics of pregnant women in the third trimester and low back pain.

## Methods

The research method used is observational analysis with a cross-sectional approach. This research activity was conducted at the Department of Obstetrics and Gynecology and Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Universitas Padjadjaran/ Dr. Hasan Sadikin General Hospital, Bandung from June to December 2017. The sample in this study were pregnant women in their third trimester who complained of lower back pain and lived in Bandung. The sampling technique used in this study was nonprobability sampling with the consecutive sampling method. In this consecutive sampling method, researchers took all subjects diagnosed with low back pain in the third trimester of pregnancy until the minimum number of subjects was met. The sample size was determined following the study's research objectives and data types in the study. The total sample was 38 patients. In this study, low back pain was described using pain intensity indicators. Pain intensity is measured using a numeric rating scale (NRS) from 0–10. A score of 0 means no pain, 1–3 means mild pain, 4–6 means moderate pain, and 7–10 means severe pain. The collected data was then processed and analyzed statistically using univariate and bivariate analysis methods. Univariate analysis aims to determine the description of the characteristics of respondents, while bivariate analysis is to determine the association between the characteristics of pregnant women in the third trimester and the intensity of low back pain using the chi-square, Mann-Whitney, and Spearman rank correlation statistical tests. The ethical clearance of the study is No. LB.04.01/ A05/EC/008/I/2018.

## Results

According to Table 1, the majority of pregnant women in the third trimester with LBP were under the age of 35 (33 of 38), had multiparous parity (25 of 38), had normal pre-pregnancy weight (21 of 38), and gained an average of 11.28 kg during pregnancy.

Pain intensity in low back pain in third-trimester pregnant women who live in Bandung is expressed on the NRS scale with a range of 0–10. According to Table 2, the median pain intensity value for pregnant women in the third trimester is 5, while the maximum pain intensity is 8.

The association between clinical characteristics of pregnant women in the third trimester and LBP pain intensity was analyzed using bivariate analysis. Table 3 shows that only age is significantly associated with LBP pain intensity, with a p-value of 0.029 ($p<0.05$) and a positive correlation. Parity is not significantly associated with pain intensity, with a p-value of

**Table 1 Characteristics of Pregnant Women**

| Variables | n=38 |
|---|---|
| Age (years) | |
| <35 | 33 |
| ≥45 | 5 |
| Parity | |
| Primiparous | 13 |
| Multiparous | 25 |
| Body weight before pregnancy | |
| Underweight | 1 |
| Normal | 21 |
| Obesity | 3 |
| Overweight | 13 |
| Weight gain during pregnancy (kg) | |
| Average (SD) | 11.28 (5.02) |
| Range | 3.70–33.00 |

**Table 2 Frequency Distribution of LBP Pain Intensity**

| | Pain Intensity |
|---|---|
| Median | 5 |
| Range | 5–8 |

**Table 3 Association between the Characteristics of Pregnant Women in the Third Trimester and LBP Pain Intensity**

| Variables | Pain Intensity | |
|---|---|---|
| | rs* | p |
| Age | 0.354 | 0.029 |
| Parity | 0.261 | 0.113 |
| Weight gain during pregnancy | −0.208 | 0.211 |

Note: *Spearman ranks correlation test, *significance p<0.05

0.113 (p>0.05), and the correlation is positive. Weight gain during pregnancy also does not have a significant association with pain intensity, with a p-value of 0.211 (p>0.05), and the correlation is negative.

## Discussion

LBP usually begins to appear in the second or 18th weeks of pregnancy and peaks in the 3rd trimester.[4,15] In this study, it was found that the median pain intensity in pregnant women in the third trimester was 5, which means moderate pain. Research by Saxena et al.[16] in India showed similar results with an average pain intensity of 4.9. Sencan et al.[17] in Turkey found a lower average of 3.7, while Gutke et al.[18] in England found a higher average of 7. The varying pain intensities in each country reflect social and cultural factors that influence the perception of LBP during pregnancy.[6]

The intensity of LBP typically increases with gestational age. Based on research conducted by Backhausen et al.[9] in Denmark, pregnant women at 32 weeks of gestation have a more excellent pain intensity score than those at 20 weeks. The median pain intensity score during 32 weeks of gestation is 4, whereas, at 20 weeks, it is 2.7.

A shift in the center of gravity causes increased LBP pain during the third trimester of pregnancy.[19] The increased uterus size during pregnancy might weaken the abdominal muscles, putting more strain on the lumbar muscles. The pelvis rotates sagittally around the second sacral segment, which serves as a fulcrum. It creates hyperlordosis, which shifts the woman's center of gravity forward and exacerbates her LBP pain.[4]

In addition, hormonal changes might cause low back pain. Estrogen, progesterone, and relaxin are prenatal hormones that influence the musculoskeletal system in preparation for childbirth.[4] During pregnancy, elevated amounts of relaxin, progesterone, and estrogen can lead to increased joint laxity. Estrogen and progesterone increase fluid and salt retention, while relaxin softens the body's ligaments, making pregnant women particularly sensitive to damage. Although the effects of relaxin are still widely debated, estrogen and progesterone are known to affect the biomechanical structure of a pregnant woman's posture by affecting connective tissue and increasing the mobility of joint capsules and spinal segments.[11]

There is currently no consensus regarding the risk factors for LBP in pregnancy. However, age has been indicated as a risk factor for LBP.[7] In this study, the majority of pregnant women in the third trimester with LBP who had a moderate pain intensity were under 35 years old (33 of 38) with a p-value of 0.029 (p<0.05) and a positive correlation. The p-value of 0.029 suggests a significant association between age and pain intensity in LBP, and the positive correlation implies that as you age, the severity of LBP pain increases. These findings are consistent with Mulati et al.'s[20] research, which suggests a correlation between age and LBP. This happens because older adults, including pregnant women, generally undergo neurophysiological changes that might increase pain threshold and decrease awareness of sensory stimuli. Aside from that, chronic disorders such as heart abnormalities, cardiovascular disease, or diabetes mellitus, which are common in the elderly, begin to impair nerve impulse transmission. This condition is exacerbated by the tendency of older people to consider pain as a natural component of the aging process and not immediately treated by health workers. Hence, the intensity of LBP pain becomes worse in older pregnant women in the third trimester.[21]

Parity has been identified as a risk factor for LBP. The results of the study showed that most pregnant women in the third trimester who experienced LBP with a median pain intensity of 5 had multiparous parity (25 of 38), with a p-value of 0.113 (p>0.05) and a positive correlation. The p-value of 0.113 indicates no significant association between parity and pain intensity in LBP. These findings are consistent with Manyozo et al.'s[7] research, which found that gravidity was

not significantly associated with the occurrence of LBP in the sample population when tested at a significance level of 5%. Backhausen et al.'s[9] research yielded different results, indicating that multiparity predicted low back pain ranging from moderate to severe. The study also found that parity has a positive correlation value, implying that the more parity, the higher the pain intensity. Previous studies indicated that the increased risk of LBP at parity has limitations because of numerous confounding factors, such as young age and a history of LBP.[15] The prevalence tended to rise with subsequent births following the first birth, but this increase mostly vanished when the age at first birth was considered.

Weight gain during pregnancy is also a risk factor for LBP. The findings revealed that the average weight gain during pregnancy among pregnant women in the third trimester who suffered LBP with moderate pain intensity was 11.28 kg, with a p-value of 0.211 ($p>0.05$) and a negative correlation. The p-value of 0.211 suggests no significant association exists between weight gain during pregnancy and pain intensity in LBP. In contrast, the negative correlation indicates that the lower the weight gain during pregnancy, the greater the pain intensity. This finding contradicts Berber and Satılmış's[22] research, which found a statistically significant difference in weight gain during pregnancy between groups of pregnant women who suffered from LBP and those who did not. Pregnant women with LBP tended to gain more weight. The difference in results is assumed to be because the weight of pregnant women who experienced the slightest weight gain had a high weight from the start, so their weight did not increase significantly during pregnancy.

Weight gain during pregnancy is common and important for fetal growth. Generally, pregnant women experience a weight gain of 11 to 15 kilograms. The increase in body weight elevates the load on the spine, causing back pain. Aside from that, increased fetal weight and uterine size contribute to LBP pain by putting more pressure on blood vessels and nerves in the back and pelvis.[23] This results in a higher prevalence of LBP in the third trimester than in the second and first trimesters.

This study has limitations, namely the lack of data on the history of LBP before pregnancy, which is one of the risk factors for LBP in pregnant women.

## Conclusions

According to the research findings, a significant association existed between age in the third trimester of pregnancy and the intensity of LBP pain. However, no significant association was found between parity and weight gain during pregnancy with the intensity of LBP pain.

## Conflict of Interest

None declared.

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## References

1. Schröder G, Kundt G, Otte M, Wendig D, Schober HC. Impact of pregnancy on back pain and body posture in women. J Phys Ther Sci. 201;28(4):1199–207.
2. Michoński J, Walesiak K, Pakuła A, Glinkowski W, Sitnik R. Monitoring of spine curvatures and posture during pregnancy using surface topography – case study and suggestion of method. Scoliosis Spinal Disord. 2016;11(Suppl 2):31.
3. Mattiuzzi C, Lippi G, Bovo C. Current epidemiology of low back pain. J Hosp Manag Health Policy. 2020;4:15.
4. Casagrande D, Gugala Z, Clark SM, Lindsey RW. Low back pain and pelvic girdle pain in pregnancy. J Am Acad Orthop Surg. 2015;23(9):539–49.
5. Chen S, Chen M, Wu X, Lin S, Tao C, Cao H, et al. Global, regional and national burden of low back pain 1990–2019: a systematic analysis of the Global Burden of Disease study 2019. J Orthop Translat. 2022;32:49–58.
6. Omoke NI, Amaraegbulam PI, Umeora OUJ, Okafor LC. Prevalence and risk factors for low back pain during pregnancy among women in Abakaliki, Nigeria. Pan Afr Med J. 2021;39:70.
7. Manyozo SD, Nesto T, Bonongwe P, Muula AS. Low back pain during pregnancy:

Prevalence, risk factors and association with daily activities among pregnant women in urban Blantyre, Malawi. Malawi Med J. 2019;31(1):71–6.
8. Fatmarizka T, Ramadanty RS, Khasanah DA. Pregnancy-related low back pain and the quality of life among pregnant women : a narrative literature review. JPHTCR. 2021;4(3):108–16.
9. Backhausen MG, Bendix JM, Damm P, Tabor A, Hegaard HK. Low back pain intensity among childbearing women and associated predictors. A cohort study. Women Birth. 2019;32(4):e467–76.
10. Daneau C, Abboud J, Marchand AA, Houle M, Pasquier M, Ruchat SM, et al. Mechanisms underlying lumbopelvic pain during pregnancy: a proposed model. Front Pain Res (Lausanne). 2021;2:773988.
11. van Benten E, Pool J, Mens J, Pool-Goudzwaard A. Recommendations for physical therapists on the treatment of lumbopelvic pain during pregnancy: a systematic review. J Orthop Sports Phys Ther. 2014;44(7):464–73, A1–15.
12. Mota MJ, Cardoso M, Carvalho A, Marques A, Sá-Couto P, Demain S. Women's experiences of low back pain during pregnancy. J Back Musculoskelet Rehabil. 2015;28(2):351–7.
13. Gutke A, Betten C, Degerskär K, Pousette S, Olsén MF. Treatments for pregnancy-related lumbopelvic pain: a systematic review of physiotherapy modalities. Acta Obstet Gynecol Scand. 201;94(11):1156–67.
14. Hall H, Cramer H, Sundberg T, Ward L, Adams J, Moore C, et al. The effectiveness of complementary manual therapies for pregnancy-related back and pelvic pain: a systematic review with meta-analysis. Medicine (Baltimore). 2016;95(38):e4723.
15. Alkaf S, Zulissetiana EF, Muslimah SU, Masturah F. Risk factors analysis of low back pain in pregnancy. JKK. 2019;6(3):116–22.
16. Saxena AK, Chilkoti GT, Singh A, Yadav G. Pregnancy-induced low back pain in Indian women: prevalence, risk factors, and correlation with serum calcium levels. Anesth Essays Res. 2019;13(2):395–402.
17. Sencan S, Ozcan-Eksi EE, Cuce I, Guzel S, Erdem B. Pregnancy-related low back pain in women in Turkey: prevalence and risk factors. Ann Phys Rehabil Med. 2018;61(1):33–7.
18. Gutke A, Boissonnault J, Brook G, Stuge B. The severity and impact of pelvic girdle pain and low-back pain in pregnancy: a multinational study. J Womens Health (Larchmt). 2018;27(4):510–7.
19. Rohmawati H, Purnani WT, Lutfiasari D, Widhi AN. The effect of pelvic rocking on back pain intensity in third trimester pregnant women. JGRPH. 2023;8(1):85–8.
20. Mulati TS, Wahyuni T, Kuswati K, Susilowati D. Factors that affect back pain in second and third trimester pregnant women. J Kebidanan Kesehat Tradis. 2022;7(1):30–41.
21. Noviyanti, Azwar Y, Santi E, Larasati DT. Faktor-faktor yang berhubungan dengan keluhan nyeri punggung bawah pada pekerja welding. Health Care. 2021;10(1):168–80.
22. Berber MA, Satılmış İG. Characteristics of low back pain in pregnancy, risk factors, and its effects on quality of life. Pain Manag Nurs. 2020;21(6):579–86.
23. Salari N, Mohammadi A, Hemmati M, Hasheminezhad R, Kani S, Shohaimi S, et al. The global prevalence of low back pain in pregnancy: a comprehensive systematic review and meta-analysis. BMC Pregnancy Childbirth. 2023;23(1):830.

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**RESEARCH ARTICLE**

# Relationship of Physical Activity and Vitamin D Levels in Elderly Women

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**Abstract**

Older adults are at risk of vitamin D deficiency, especially in older women, due to a decrease in the hormone estrogen, which causes decreased bone density and increased risk of fractures. There is a relationship between vitamin D deficiency and lack of physical activity due to the storage of vitamin D in adipose tissue. Physical activity was measured using the International Physical Activity Questionnaire (IPAQ). The aim was to determine the relationship between physical activity and vitamin D in elderly women. This cross-sectional analytic observational study was conducted at Nursing Home X in Surabaya city from May to August 2023. The variables were physical activity (measured by IPAQ to estimate physical activity levels) and vitamin D level (measured by VIDAS® instrument to determine 25(OH) levels). The subjects were all women aged ≥60 years. The sampling technique uses the purposive sampling method. For an analysis of the relationship between physical activity levels and vitamin D levels, the Pearson correlation test was used with a ratio data scale. This research received 49 respondents. The average physical activity level was 1316.82±720.90, and most respondents had a moderate physical activity level of 44.90%. Vitamin D levels were ≤30 ng/ml for 46 respondents (93.87%). The results of the Pearson correlation test were obtained with a $r_{value}$=0.089 and a significance value (Sig.) of 0.542. There was no significant correlation between physical activity and vitamin D levels. Other factors such as age, health conditions, and vitamin D intake can affect the results. Further research can be developed into measurements to examine physical activity in old age.

**Keywords:** IPAQ, elderly woman, nursing home, physical activity, vitamin D

## Introduction

Vitamin D deficiency is a common global health problem with high prevalence, especially in South Asia countries. Vitamin D mainly regulates calcium and phosphorus metabolism and promotes bone growth.[1] Older adults are at risk of vitamin D deficiency because vitamin D production and metabolism change with age due to various factors, such as reduced sun exposure and skin production capacity.[2] Physically, geriatrics are less active and have poor nutritional status. In addition, there are physiological processes that exacerbate the decrease in vitamin D levels in the body, such as decreased production of vitamin D in the skin after sun exposure caused by skin atrophy, eating foods that are low in vitamin D, impaired gastrointestinal absorption, and decreased production $1,25(OH)_2D$ in the kidney.[3] Vitamin D deficiency is prevalent in postmenopausal women, and this undoubtedly exacerbates the risk of cardiovascular disease and menopause-related dyslipidemia. Estrogen levels drop significantly as women go through menopause, and a lack of estrogen weakens bones. Vitamin D helps the body absorb and use calcium to maintain bone structure. 25-hydroxyvitamin D [25(OH)D] serves the purpose of calcium absorption and appears to be hormonally sensitive. Vitamin D deficiency is associated with low bone mass and increased fracture risks. Menopausal women are at risk of decreased bone density and increased risk of fractures due to decreased estrogen levels,[1] reduced vitamin D synthesis in the skin, or changes in body composition relevant to vitamin

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D status and physiology.[4] Vitamin D deficiency is defined as vitamin D levels below 20 ng/ml (50 nmol/liter), and vitamin D deficiency is 21–29 ng/ml (525–725 nmol/liter). Vitamin D deficiency can also be a risk factor for diseases such as immune system diseases and infectious diseases caused by coronavirus disease 2019 (COVID-19).[5] Research by Pereira et al.[6] reported that low vitamin D levels were associated with COVID-19.

Research by Cui et al.[5] shows that 15.7% of the global adult population has vitamin D deficiency. Vitamin D deficiency can occur in all age groups, including menopausal women. Indonesia currently has 7.4% of menopausal women from the total population, and in 2020, it is estimated to reach 11.54% with an average age of menopause of 49 years. According to Statistics Indonesia, in 2015, the number of women in Indonesia who entered menopause reached 21.22 million. It is estimated that by 2025, there will be 60 million menopausal women.[7] Another study on the incidence of vitamin D deficiency in Indonesian women showed that 95% of them (148 subjects out of a total of 156) had vitamin D deficiency.[8]

There is a relationship between vitamin D deficiency and lack of physical activity due to the storage of vitamin D in adipose tissue due to its high fat solubility and decreased exposure to sunlight in obese subjects due to limited physical activity and limited mobility. It is proven that physical activity increases glucose, calcium, and vitamin D metabolism and reduces body weight by increasing lipolysis.[9] Someone who does high physical activity will be associated with increased vitamin D concentration. Suppose someone does less physical activity (low physical activity). In that case, it will cause a decrease in vitamin D concentration, while someone with high physical activity will most likely be associated with optimal vitamin D levels.[10] Vitamin D can also help improve the body's performance in physical activities in populations at risk of vitamin D deficiency. Previous research by Nascimento et al.[11] found a significant increase in levels and improvements in the body, so there was an interaction between vitamin D levels and physical activity in maintaining body performance.

Measurement of physical activity in this study used a questionnaire, namely the extended version of the International Physical Activity Questionnaire (IPAQ), which is used to estimate physical activity levels. Data on the relationship between physical activity and vitamin D levels in Indonesia have been collected by Suryadinata et al.,[12] conducted at Public Health Center Taman, Sidoarjo district, East Java, in March–July 2017. The results showed geriatrics with obesity and non-obesity had similar levels of physical activity, but vitamin D status in obesity tended to be lower than non-obese. However, similar studies have never been conducted on elderly women in nursing homes. These data would greatly assist health workers in improving the quality of life of these patients. This research aimed to determine the relationship between physical activity and vitamin D in elderly women.

## Methods

This study used analytic observational research with a cross-sectional design. Data was collected from May 2023 to August 2023 at Nursing Home X in Surabaya city. The Institutional Ethical Committee of the University of Surabaya approved the study, 207/KE/VIII/2023.

This study's population was all women aged ≥60 who had experienced menopause in Nursing Home X in Surabaya city. The samples taken in this study were all populations that met the inclusion criteria. The sampling technique used is purposive sampling, which meets the requirement of not having physical limitations such as motor disorders.

Each respondent will be interviewed to fill out the IPAQ and take blood samples to check vitamin D levels. A phlebotomist from Pramita Laboratorium will take a blood sample and test it at an accredited laboratory.

Blood sampling was carried out simultaneously during the day by the phlebotomist, taking into account the time of blood sampling, which can affect vitamin D levels.[13] After blood collection, the laboratory will process the blood by centrifuging to obtain serum, which will then be tested for vitamin D levels using VIDAS® 25-OH Vitamin D Total (VITD), which is an automatic quantitative test used on the VIDAS® instrument to determine 25(OH) levels. D total in human serum or plasma with the enzyme-linked fluorescence assay (ELFA) technique.

The method for measuring physical activity uses the modified IPAQ. The physical activity questionnaire contains 12 questions about light,

moderate, and vigorous physical activity activities. Respondents were interviewed, and then results were obtained, which categorized respondents into mild, moderate, and vigorous categories using the metabolic equivalent of task (MET). Physical activity is divided into three categories, namely the mild category (<600 MET minutes/ week), the moderate category (600–1,500 MET minutes/week), and the vigorous category (>1,500 MET minutes/week). This questionnaire contains questions about the type of activity, duration, and frequency of a person doing physical activity in the last 7 days. Physical activity can be measured by measuring the amount of energy expended/needed in each minute of activity. According to the IPAQ assessment protocol, MET-minutes/week for a given activity (walking moderate-intensity activity or vigorous-intensity activity) is calculated by multiplying the MET value of the given activity (3.3 for walking, 4.0 for moderate-intensity activity, and 8.0 for high-intensity activities) according to hours spent in a particular activity, for example walking at work = 3.3 × minutes walking × days walking at work. MET-minutes/week = (MET × activity minutes × days). Information: MET-minutes/week = METs produced in one week MET = MET value based on activity level minutes = duration of activity in one day days = number of days in one activity.[14,15] Results are presented as estimates of energy expenditure in metabolic equivalent minutes per week (MET-hours/week).[16,17] The metabolic equivalent of task, or MET, is valid for describing energy expenditure from certain activities. MET is the ratio of the rate of energy expended during activity to the rate of energy expended at rest.[18]

Before use, the questionnaire (IPAQ)[11,16,19] must be used as a research instrument, and a validity test must first be carried out, consisting of content and construct validation. Content validity through rational analysis or expert judgment by a panel to ensure that measurements are representative. Meanwhile, construct validity refers to the extent to which a measuring instrument shows results following theory. Reliability shows that the questionnaire is reliable and consistently provides the same results when measured twice or more with the same instrument. Cronbach's alpha value was >0.6, so the research instrument would be reliable.

The relationship between physical activity levels and vitamin D levels was analyzed using the Pearson correlation test, with the data scale being the ratio. The data was tested for normality using the Shapiro-Wilk test; if the p-value>0.05, it was concluded that the data was normally distributed. The relationship test used the Pearson correlation test; if the p-value<0.05, it can be concluded that there is a significant relationship between physical activity and vitamin D levels.

## Results

This research involved 49 geriatric women as respondents. Data were collected using physical activity questionnaire interviews and vitamin D serum tests. The study involved elderly women aged 60 years and over. The highest number of high-risk elderly women >70 years was 43 respondents, while the rest were in the 60–70-year age group.

Regarding body mass index (BMI) characteristics, most respondents were of normal weight, and 34 respondents had an average standard deviation of 21.58±2.16 kg/m$^2$. Most of them had no disease history (Table 1).

Based on the data in Table 2, respondents' most common physical activity profile was exercise (44 respondents) and walking 100 m (39 respondents). At the same time, physical activities that were only done by some respondents were cooking and cleaning the garden or the like. Seven respondents carry out physical activity in the mild category, 22 in the moderate category,

**Table 1 Frequency Distribution of Respondent Characteristics**

| Characteristics | n=49 |
|---|---|
| Age (years) | |
| 60–70 | 6 |
| >70 | 43 |
| BMI (kg/m$^2$) | |
| Very thin (<16) | 6 |
| Skinny (16–18.4) | 6 |
| Normal (18.5–24.9) | 34 |
| Overweight (25–29.9) | 3 |
| Obesity (>30) | 0 |
| Medical history | |
| Type 2 diabetes mellitus | 5 |
| Hypertension | 10 |
| Osteoarthritis | 7 |
| Osteoporosis | 3 |
| There isn't any | 27 |

**Table 2 Profile of Respondents' Physical Activity Questionnaire Answers**

| No. | Types of Physical Activity | Answer (Minutes) | n=49 |
|---|---|---|---|
| 1 | Walk 100 m | 5 | 1 |
| | | 10 | 5 |
| | | 15 | 6 |
| | | 20 | 1 |
| | | 30 | 23 |
| | | 60 | 3 |
| | | Didn't do | 10 |
| 2 | Walk more than 100 m | 10 | 2 |
| | | 15 | 3 |
| | | 60 | 12 |
| | | Didn't do | 32 |
| 3 | Cook | Didn't do | 49 |
| 4 | Wash clothes by hand | 10 | 4 |
| | | 15 | 2 |
| | | 20 | 2 |
| | | 30 | 9 |
| | | Didn't do | 32 |
| 5 | Cleaning room | 5 | 1 |
| | | 10 | 5 |
| | | 15 | 1 |
| | | 30 | 2 |
| | | Didn't do | 40 |
| 6 | Exercise | 10 | 7 |
| | | 15 | 13 |
| | | 20 | 4 |
| | | 25 | 6 |
| | | 30 | 4 |
| | | 45 | 3 |
| | | 60 | 8 |
| | | Didn't do | 5 |
| 7 | Cleaning the garden | Didn't do | 49 |
| 8 | Gardening/farming | 30 | 2 |
| | | Didn't do | 47 |
| 9 | Sew with hand | 10 | 2 |
| | | 15 | 3 |
| | | 20 | 1 |
| | | 60 | 4 |
| | | Didn't do | 39 |
| 10 | Sewing by machine | 60 | 1 |
| | | Didn't do | 48 |
| 11 | Read | 10 | 4 |
| | | 15 | 2 |
| | | 20 | 1 |
| | | 30 | 4 |
| | | 60 | 4 |
| | | Didn't do | 34 |
| 12 | Watch TV | 5 | 1 |
| | | 10 | 3 |
| | | 15 | 3 |
| | | 20 | 1 |
| | | 30 | 6 |
| | | 60 | 2 |
| | | Didn't do | 33 |

and 20 in the vigorous category. The average physical activity level was 1316.82±720.90, and most respondents had a moderate physical activity level of 44.90% (Table 3).

Vitamin D levels were measured during the research by taking blood samples from each respondent. In Table 4, the results showed that vitamin D levels[16,17] were <20 ng/ml for 27 respondents, vitamin D levels were 20–30 ng/ml for 19 respondents, and vitamin D levels were >30 ng/ml for as many as three respondents.

Based on Table 5, the results showed that deficiency (<20 ng/ml) for 27 respondents (mean±SD=17.40±2.07),insufficiency(20–30ng/ml) for 19 respondents (mean±SD=23.64±2.42), and normal level (>30 ng/ml) for three respondents (mean±SD=31.33±1.15). The total mean±SD was 18.41±6.60. In the mild physical activity category, two respondents had vitamin D levels <20 ng/ml, and 20–30 ng/ml for five respondents. In the category of moderate physical activity, 14 respondents had vitamin D levels <20 ng/ml, and 20–30 ng/ml as many as eight respondents. In the category of vigorous physical activity, 11 respondents had vitamin D levels <20 ng/ml, six respondents had 20–30 ng/ml, and three had >30 ng/ml.

The normality test results are as follows: In the Shapiro-Wilk normality test, a significance value (Sig.) was obtained, which was 0.133 for physical activity and 0.94 for vitamin D levels. Because both data have a significance value of >0.05, it can be concluded that the physical activity data and vitamin D levels were usually distributed. The results of the Pearson correlation test are as follows: obtained an $r_{value}$ (Pearson correlation) of 0.089 and a significance value (Sig.) of 0.542. Based on a significance value (α) >0.05, it means there is no significant correlation between physical activity and vitamin D levels (Figure).

## Discussion

This research involved 49 geriatric women as respondents. Since this study only involved 49 respondents, which may need to be more representative of a larger group, the results cannot be generalized widely and only to elderly women with similar characteristics.

Based on age, the highest number in the group of high-risk elderly women, namely 70 years and over, was 43 respondents, while in the 60–70

**Table 3 Physical Activity Level of Respondents**

| Category Physical Activity | n=49 | Mean±SD | Total Mean±SD |
|---|---|---|---|
| Mild (<600) | 7 | 312.21±162.37 | 1,316.82±720.90 |
| Moderate (600–1,500) | 22 | 1,038.75±401.43 | |
| Vigorous (>1,500) | 20 | 2,074.17±581.39 | |

**Table 4 Presentation of Data on Vitamin D Level Values**

| Vitamin D Level (ng/ml) | n=49 | Mean±SD | Total Mean±SD |
|---|---|---|---|
| Deficiency (<20) | 27 | 17.40±2.07 | 18.41±6.60 |
| Insufficiency (20–30) | 19 | 23.64±2.42 | |
| Normal (>30) | 3 | 31.33±1.15 | |

**Table 5 Cross Tabulation of Vitamin D Levels with Physical Activity**

| Physical Activity | Number of Respondents based on Vitamin D Level Value | | | Total n=49 |
|---|---|---|---|---|
| | Deficiency n=27 | Insufficiency n=19 | Normal n=3 | |
| Mild (<600) | 2 | 5 | 0 | 7 |
| Moderate (600–1,500) | 14 | 8 | 0 | 22 |
| Vigorous (>1,500) | 11 | 6 | 3 | 20 |

Note: deficiency (<20 ng/ml), insufficiency (20–30 ng/ml), normal (>30 ng/ml)

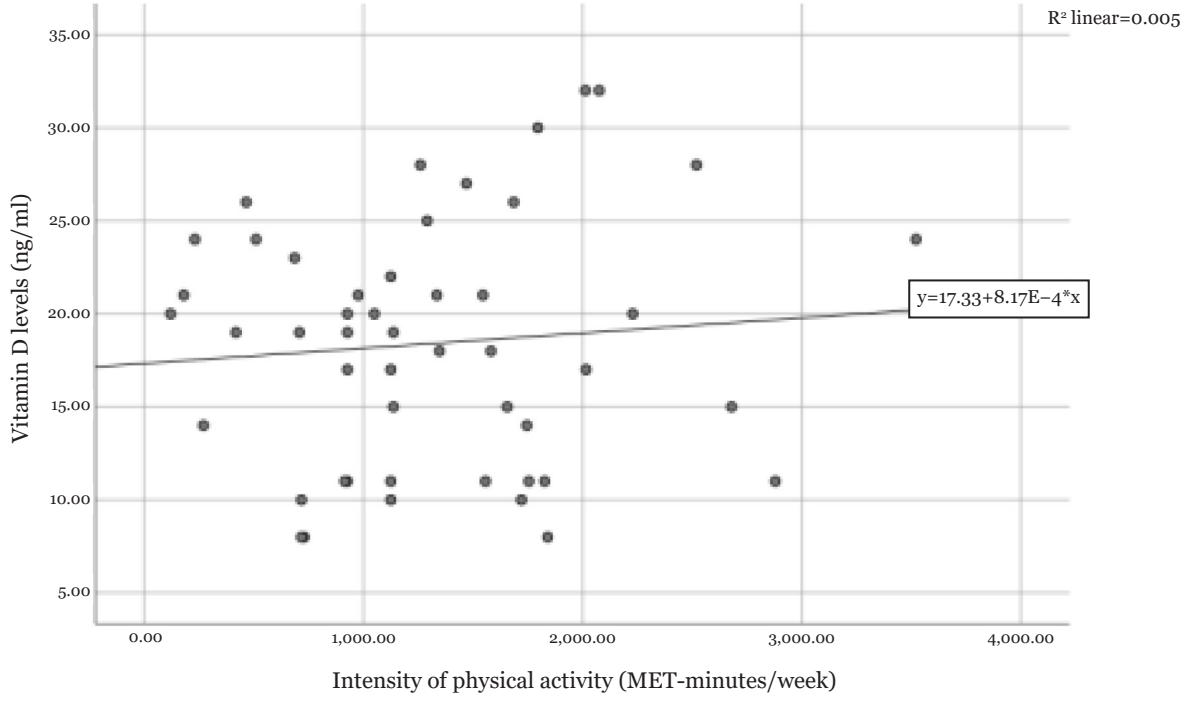


**Figure Distribution of Physical Activity Data and Vitamin D Levels**

year age group, there were only six respondents. Uneven age distribution may affect the results, especially physiological changes in different age groups. Age can affect vitamin D levels, such as a decreased vitamin D metabolism in old age. A decrease in calcium absorption causes this, the presence of intestinal resistance to absorption of circulating calcium $1,25(OH)_2D$, a decrease in kidney function resulting in a reduction of $1,25(OH)_2D$ production in the kidneys, and a decrease in vitamin D production in the skin, nine caused by decreasing the concentration of 7-dehydrocholestrol in the epidermis which can reduce the formation of vitamin D3. So aging can also cause calcium deficiency, hyperparathyroidism, increased bone loss, and osteoporosis.[20,21]

The majority of respondents (27 people) had no history of illness, while only several suffered from diabetes, hypertension, or osteoarthritis. This imbalance in health characteristics can affect physical activity and vitamin D levels. History of disease can affect vitamin D levels, including type 2 diabetes mellitus. The relationship between vitamin D deficiency and insulin resistance can develop through inflammation because vitamin D deficiency is associated with increased inflammatory markers. In addition, genetic polymorphisms of vitamin D-related genes may predispose to impaired glycemic control and type 2 diabetes. Epidemiological studies suggest an association between low serum 25-hydroxyvitamin D3 (25(OH)D3) concentrations and an increased risk of metabolic syndrome and type 2 diabetes. The increase in fat mass partly explains it.[20,21]

Most respondents had low vitamin D levels (<20 ng/ml), and only a few had levels above 30 ng/ml. This uneven distribution may limit statistical power for analysis and comparison between groups. The research showed no significant correlation between physical activity and vitamin D levels. It differs from previous research by Trovato et al.,[22] which stated that higher dietary intake of vitamin D and sunlight exposure are associated with a lower likelihood of having high perceived stress among physically active individuals. These results may be related to previous research by Zhang and Cao,[23] that endurance exercise can significantly increase serum 25(OH)D levels in vitamin D-deficient people but has no significant effect on vitamin D-sufficient people. The effects of exercise on 25(OH)D levels in the circulation may depend on factors such as vitamin D nutritional status, exercise type and intensity, and sex.

Physical activity can be measured with a tool, namely an accelerometer, pedometer, and self-report questionnaire (IPAQ) instrument. This research uses a self-report questionnaire because it has the advantage of cheaper costs, does not burden respondents, and obtains the flow of daily physical activity from respondents. Still, the self-report questionnaire has areas for improvement, such as less accuracy and reliability, because the data is based only on respondents' memories.[24]

This study used a modified IPAQ to measure the intensity of physical activity. The IPAQ is the most frequently and commonly used measurement tool in measuring physical activity levels. However, the questionnaire has limitations because it is based on respondents' memories. As in previous research by Cleland et al.,[16] results showed the majority of older adults under-report their level of moderate-to-vigorous physical activity and sedentary behavior when completing the IPAQ, and the linear relationship above the mean shows an error from under to over-reporting as the mean increases.

Suggestions for future research include expanding research on older-specific physical activity measures, such as previous research by VandeBunte et al.,[25] where actigraphy metrics are not available, standard self-report measures of PA are used in older adults to capture more structured activity and exercise routines (e.g., duration, type of exercise). The Community Healthy Activities Model Program for Seniors (CHAMPS) and the Physical Activity Scale for the Elderly (PASE) are two widely used self-report PA questionnaires in older adults. Fitbit monitors have demonstrated inter-device reliability with other actigraphy monitors. Results showed that Fitbit's step counts showed a stronger association with PASE, while Fitbit's burned calories were more strongly associated with CHAMPS-MET. Fitbit results had more consistent convergence with relevant outcomes of interest (e.g., brain and cardiometabolic health indices) compared to subjective measures; however, considerable heterogeneity in these associations was observed.

Most respondents had moderate physical activity levels, while only a few were categorized as mild or vigorous. This imbalance may limit

the broad analysis of the relationship between physical activity and vitamin D. Based on the distribution of physical activity profiles; two physical activities are often done: gymnastics and walking 100 meters. Regarding walking activity alone, many age-related changes contribute to the increased energy requirements for walking, which can be two to four times that of a healthy adult. Age-related biomechanical factors, such as a flexed trunk, limited hip extension, and reduced ankle movement in gait, result in less use of stored passive energy and greater demands on muscle activity. Age-related neuromuscular factors alter efficient muscle recruitment patterns and the timing of limb movements, resulting in inefficient walking due to compensating for body changes.[20] Several factors can influence a person in carrying out physical activity, including:[14,15]

(1) Intrinsic factors. This factor is more directed at a person's inner motivation for activities. A person with good feelings will do more activities, while a person with bad feelings will do less. Even though the questionnaire has been validated, a person's motivation to move can also influence a person's physical activity.

(2) Environmental factors. The environment can influence physical activity, and weather that is too hot or rainy can be a barrier. Changing environmental weather conditions can also affect a person's ability to carry out physical activities, mainly because most respondents' physical activities are carried out in open areas, which the weather and environment will significantly influence.

(3) Physical consideration factors. A person's body condition can influence their physical activity. They will do physical activity if they feel their body is in good condition, but they will not if they feel tired or injured. In this study, activity restrictions are one of the requirements for research subjects so that the influence of this factor can be minimized.

(4) Routine factor. In the routine factor, a person carries out daily physical activity and/or other demands. External factors such as sun exposure, dietary intake, or, although these factors can significantly affect vitamin D levels.

During the research, vitamin D levels were measured to determine vitamin D levels in elderly women by taking blood samples from each research respondent. From a total of 49 elderly women, the majority of vitamin D levels <20 ng/ml were obtained by 27 respondents (55%) with an average of 17.40 ng/ml.

Blood samples were collected only once. This may need to be revised to adequately reflect the variation in vitamin D levels, which can change depending on daily conditions, such as sun exposure and diet. Daily variation was minimized by collecting samples at the same time of day. Blood sampling was carried out simultaneously during the noonday by the phlebotomist, taking into account the time of blood sampling, which can affect vitamin D levels.[13]

This technique can cause bias because the sample is selected based on specific criteria and does not represent the entire population of elderly women. As a result, the study's results may be challenging to apply to all older adults. To minimize the influence of vitamin D variations, several factors that can affect vitamin D variations in the respondent's body condition are minimized by not having significant changes in these factors from one month earlier (such as medication, health conditions, and lifestyle). Several factors can influence vitamin D levels, including the following:[20,21]

(1) Body mass index (BMI). Vitamin D is a fat-soluble vitamin, and people with a BMI >30 kg/m$^2$ tend to have higher fat than those with a normal BMI. The association between vitamin D deficiency and overweight and obese patients may be explained by the storage of vitamin D in adipose tissue due to its high-fat solubility and decreased exposure to sunlight in obese subjects due to limited physical activity and mobility.

(2) Lack of intake. All age-related changes in vitamin D metabolism are magnified if there is concomitant vitamin D deficiency, as it limits the supply of substrates for 25OHD and, ultimately, 1,25$(OH)_2$D. Substrate deficiency is a common problem in older people and is essential to recognize because it can be prevented and treated. There may be a vitamin D deficiency either from diet or from lack of sunlight, and a subsequent decrease in serum 25OHD limits 1,25$(OH)_2$D production, especially if there is also renal dysfunction. Serum 1,25$(OH)_2$D levels decrease when serum 25OHD levels fall below 10 ng/mL in young and older adults.

## Conclusions

The correlation test results between physical

activity and vitamin D levels in elderly women showed no significant correlation. Other factors affecting the research results include age, which has a wide range of variations, health conditions, and vitamin D intake. Further research can develop measurements specifically for examining physical activity in old age.

## Conflict of Interest

The authors declare no conflict of interest.

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## References

1. Mei Z, Hu H, Zou Y, Li D. The role of vitamin D in menopausal women's health. Front Physiol. 2023;14:1211896.
2. Giustina A, Bouillon R, Dawson-Hughes B, Ebeling PR, Lazaretti-Castro M, Lips P, et al. Vitamin D in the older population: a consensus statement. Endocrine. 2023;79(1):31–44.
3. Borowicz W, Ptaszkowski K, Ptaszkowska L, Rosińczuk J, Murawska-Ciałowicz E. Association between serum vitamin D levels and physical outcomes of patients who underwent rehabilitation following ischemic stroke. Med Sci Monit. 2023;29:e940115.
4. Liu W, Wu Z, Zhu D, Chen G, Yan G, Zhang S, et al. Vitamin D and lipid profiles in postmenopausal women: a meta-analysis and systematic review of randomized controlled trials. Front Mol Biosci. 2021;8:799934.
5. Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y. Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: a pooled analysis of 7.9 million participants. Front Nutr. 2023;10:1070808.
6. Pereira M, Dantas Damascena A, Galvão Azevedo LM, de Almeida Oliveira T, da Mota Santana J. Vitamin D deficiency aggravates COVID-19: systematic review and meta-analysis. Crit Rev Food Sci Nutr. 2020;62(5):1308–16.
7. Widyantari NPS, Wijaya IPA, Susila IMDP. Hubungan tingkat pengetahuan tentang menopause dengan kecemasan menghadapi menopause pada ibu Pembinaan Kesejahteraan Keluarga. Caring. 2019;3(2):56–9.
8. Sari DK, Alrasyid DH, Nurlndrawaty L, ZulkifIi L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. Malaysian Journal of Nutrition. 2014;20(1):63–70.
9. Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D sources, metabolism, and deficiency: available compounds and guidelines for its treatment. Metabolites. 2021;11(4):255.
10. Wiciński M, Adamkiewicz D, Adamkiewicz M, Śniegocki M, Podhorecka M, Szychta P, et al. Impact of vitamin D on physical efficiency and exercise performance—a review. Nutrients. 2019;11(11):2826.
11. Nascimento NAP, Moreira PFP, Carvalho VA, Aragão L, Marin-Mio RV, Lazaretti-Castro M, et al. Effect of vitamin D Level and physical exercise on the physical performance and functional test results in elderly women. J Geriatr Med Gerontol. 2019;5(1):061.
12. Suryadinata RV, Lorensia A, Tangkilisan EC. Effect of physical activity and vitamin D status on geriatrics obesity. GMHC. 2019;7(1):1–6.
13. Abu Jadayil S, Abu Jadayel B, Takruri H, Muwalla M, McGrattan AM. Study of the fluctuation of serum vitamin D concentration with time during the same day and night on a random sample of healthy adults. Clin Nutr ESPEN. 2021;46:499–504.
14. Li J, Huang Z, Si W, Shao T. The effects of physical activity on positive emotions in children and adolescents: a systematic review and meta-analysis. Int J Environ Res Public Health. 2022;19(21):14185.
15. Ho JY, Lam HYC, Huang Z, Liu S, Goggins WB, Mo PKH, et al. Factors affecting outdoor physical activity in extreme temperatures in a sub-tropical Chinese urban population: an exploratory telephone survey. BMC Public Health. 2023;23(1):101.
16. Cleland C, Ferguson S, Ellis G, Hunter RF. Validity of the International Physical Activity Questionnaire (IPAQ) for assessing moderate-to-vigorous physical activity and sedentary behaviour of older adults in the United Kingdom. BMC Med Res Methodol. 2018;18(1):176.

17. Sember V, Meh K, Sorić M, Starc G, Rocha P, Jurak G. Validity and reliability of International Physical Activity Questionnaires for adults across EU countries: systematic review and meta analysis. Int J Environ Res Public Health. 2020;17(19):7161.
18. Khan SR, Claeson M, Khan A, Neale RE. The effect of physical activity on vitamin D: a systematic review and meta-analysis of intervention studies in humans. Public Health Pract (Oxf). 2024;7:100495.
19. Heydenreich J, Schutz Y, Melzer K, Kayser B. Comparison of conventional and individualized 1-MET values for expressing maximum aerobic metabolic rate and habitual activity related energy expenditure. Nutrients. 2019;11(2):458.
20. Wang LK, Hung KC, Lin YT, Chang YJ, Wu ZF, Ho CH, et al. Age, Gender and season are good predictors of vitamin D status independent of body mass index in office workers in a subtropical region. Nutrients. 2020;12(9):2719.
21. Lorensia A, Suryadinata RV, Arganitya GN. Relationship of vitamin D intake with obesity in adolescents. GMHC. 2022;10(2):104–10.
22. Trovato B, Godos J, Varrasi S, Roggio F, Castellano S, Musumeci G. Physical activity, sun exposure, vitamin D intake and perceived stress in Italian adults. Nutrients. 2023;15(10):2301.
23. Zhang J, Cao ZB. Exercise: a possibly effective way to improve vitamin D nutritional status. Nutrients. 2022;14(13):2652.
24. Lorensia A, Suryadinata RV, Inu IA. Comparison of vitamin D status and physical activity related with obesity in student. J Appl Pharm Sci. 2022;12(4):108–18.
25. VandeBunte A, Gontrum E, Goldberger L, Fonseca C, Djukic N, You M, et al. Physical activity measurement in older adults: wearables versus self-report. Front Digit Health. 2022;4:869790.

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**RESEARCH ARTICLE**

# Ergonomic Risk Level of Work Posture in Leather Shoes Industry

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**Abstract**

Posture while working will influence work results and have a health impact on workers. Rapid upper limb assessment (RULA) is a method for analyzing work posture on the job using the upper part of the body. This research examines the risk level of work postures in the leather shoe industry. This research was conducted in the leather shoe industry located in Semarang. This research uses the RULA method to assess the risk level of work postures. Body area measured by RULA was divided into two groups, namely group A (arms, forearms, hands) and group B (neck, body). The RULA score is obtained based on observing the worker's body posture, including neck, arms, back, legs, and load. The observed scores are then converted into final RULA scores. It was found that the body posture of workers in the leather industry was at a score of 6 and 7. This score has implications for the need for further research regarding the body posture of the following workers. Work posture correction must be done immediately to prevent further health impacts.

**Keywords:** Ergonomics risk level, RULA, work posture

## Introduction

One of the potential hazards workers face in the leather shoe industry is the potential for ergonomics, primarily related to work postures. Work in the leather shoe industry is done in a sitting position. Sitting in a non-ergonomic position will trigger bone and skeletal muscle complaints. When work is carried out in a natural posture, weight is balanced on both legs without bending and twisting the torso, and it will reduce fatigue and increase productivity. The sitting position looks more comfortable than other postures while working. However, sitting for long periods will also cause fatigue. Therefore, sitting in a comfortable chair with a backrest supporting the back well will reduce fatigue. Ergonomic risks are common in the modern world of work. Musculoskeletal disorders (MSDs) are the most frequently reported work-related health problems. This causes more lost work days than any other type of health problem in some countries. MSDs represent 40% of global compensation costs resulting from occupational accidents and illnesses worldwide. The characteristics of modern work, such as the fast pace, tight deadlines, lack of control over the pace of work, and a culture of long working hours, can cause stress and ergonomic risks that are more common than ever. As a result, muscle pain, mental disorders such as anxiety and depression, and cardiovascular disease now impact more of the working population than ever before.[1]

Research conducted in the manufacturing industry showed a relationship between work posture and complaints of low back pain (p=0.047). Workers complained of uncomfortable work postures and low back pain in the category of severe crippled disability.[2]

Work posture and fatigue are essential determinants of work musculoskeletal disorders (WMSDs) found in a study on minibus drivers in Nigeria in addition to work stress, work duration, and work frequency. This study recommends that ergonomics training be conducted to reduce the incidence of WMSDs in this population.[3]

To prevent the occurrence of occupational disorders or diseases, it is necessary to identify all risk factors that occur during work. Once determined, preventive measures must be determined to prevent, reduce, or eliminate the complaint.[4]

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Workers in the shoe industry exhibit several awkward work postures, such as hunched backs, sitting on the floor, poor foot position, or sitting in chairs that do not suit their bodies. Based on previous research, these postures can trigger health complaints such as musculoskeletal disorders. Therefore, it is necessary to assess workers' posture in this industry. So, this research aims to analyze the risk level of work postures in the leather shoe industry.

## Methods

This descriptive research was conducted in the leather shoe industry in Semarang using purposive sampling methods. There were 43 workers involved in this research. However, only five workers had their work postures observed since they demonstrated similar postures in the same production step. They represent workers at every stage of the job. The postures observed were all work postures in each production process in the industry.

To assess rapid upper limb assessment (RULA), researchers took photos of workers while doing their work from a side angle. Using a protractor, the angles required for evaluation in the RULA sheet were measured through photos. The worker's dynamic movement was also observed. This measure was then converted to a RULA score and interpretation (available in the RULA sheet).[5]

The research protocol has been approved, and an ethical clearance certificate Number 472/EA/KEPK-FKM/2019 has been received.

## Results

Table 1 shows that 40 of 43 workers were in the old age category. About 27 of 43 workers were in the new work period category. Based on the study's results, it was found that work in the leather shoe industry was carried out for 8 hours every day with 1 hour of rest. The workers' work posture was sitting on a small chair without a backrest. There are even workers who sit on the floor while doing their jobs. Such a work posture could be more ergonomic and more comfortable for workers. The workplace layout needs to be more tidy. Some unused objects are still visible around the workplace. This makes it difficult for workers to carry out their work, and sometimes, some work equipment may be difficult to find because of the large number of items mixed with unused items.

**Table 1 Characteristics of Respondents**

| Characteristics | n=43 |
|---|---|
| Age (years) | |
| >30 | 40 |
| ≤30 | 3 |
| Work period (years) | |
| <5 | 27 |
| ≥5 | 16 |

Based on Figure 1, it is known that workers' posture when cutting shoe materials is according to the pattern in a sitting posture, bent back (angle a), and head bowed position. This posture puts extra pressure on the lower back and neck. The assessment of this posture showed that the upper arm, lower arm, and wrist scores were 2, 1, and 3, respectively. Adding muscle score one and load score 1, the total wrist and arm score was 4. For the neck and trunk score, each section scored 3, and the leg score was 1. This score, added to section B's muscle and load score, became 7. So, the final score for this posture was 6. It can be interpreted from the RULA sheet that further investigation and improvement of posture is needed immediately, considering that this type of work posture looks like a highly bent back.

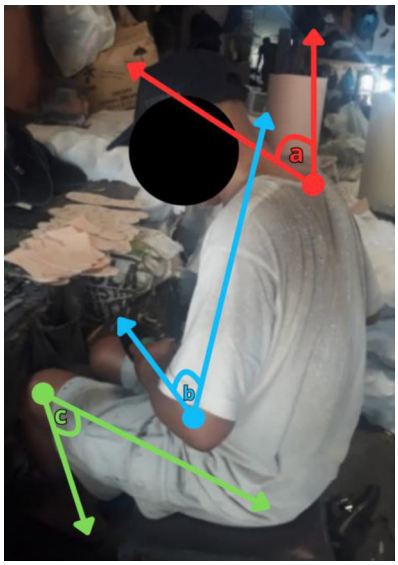


**Figure 1 Process of Cutting according to the Pattern**

The assessment of this posture (Figure 2) showed that the upper arm, lower arm, and wrist scores were 2, 1, and 3, respectively. Adding by muscle score and load score 2, the total wrist and arm score was 6. For the neck and trunk score, each section gained a score of 3; besides, each section scored 3. Besides, each section scored 3, and the leg score was 1. This score, added to section B's muscle and load score, became 6. So, the final score for this posture was 7. It can be interpreted from the RULA sheet that further investigation or research is needed to determine the impact that can arise from this posture. In addition, a change in work posture will likely be necessary given the very non-ergonomic work posture.

The work posture in Figure 3 showed that the upper arm, lower arm, and wrist scores were 2, 1, and 3, respectively. Adding muscle score one and load score 1, the total wrist and arm score was 5. For the neck and trunk score, each section scored 3 and 2, respectively; each section scored 3 and 2, respectively, and the leg score was 1. This score, added to section B's muscle and load score, became 5. So, the final score for this posture was 6. It can be interpreted from the RULA sheet that further investigation is needed regarding the posture, and the posture must be corrected immediately. It can be seen in the picture that workers are very uncomfortable in their sitting conditions; workers have to fold their legs for a long time. Of course, this will most likely cause complaints such as aches, cramps, and tingling. If the sitting posture is not immediately corrected, workers also tend to experience fatigue quickly.


**Figure 3 Gluing and Pasting the Sole**

The assessment of this posture in Figure 4 showed that the upper arm, lower arm, and wrist scores were 1, 1, and 3, respectively. Adding muscle score one and load score 1, the total wrist and arm score was 4. For the neck and trunk score, each section scored 3, and the leg score

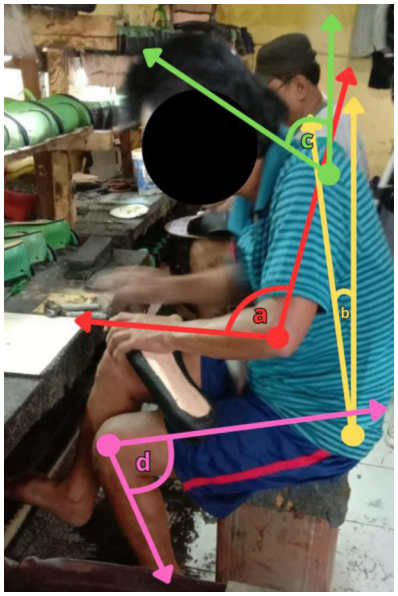


**Figure 2 Installation on A Shoe Mold**

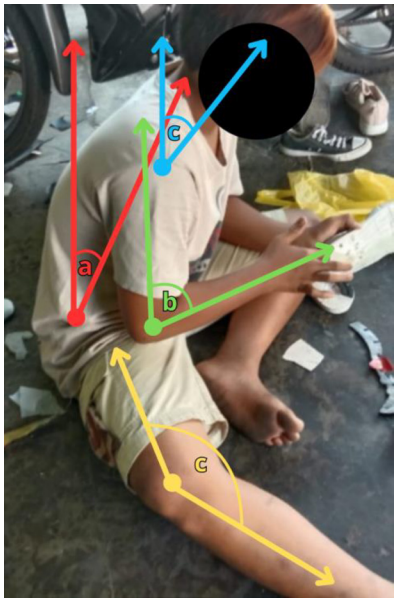


**Figure 4 Outsole Installation**

was 1. This score, added to section B's muscle and load score, became 6. So, the final score for this posture was 6. So, further investigation is needed in this case, as well as improving the work posture to make it more ergonomic. It can be seen in the picture that workers do their work by sitting on the floor without chairs or cushions. This work posture will make workers feel very uncomfortable and trigger complaints or health problems, such as knee pain and back and neck pain, and they tend to get tired quickly.

The worker in Figure 5 was doing his job with a bent posture because he was sitting on the floor with his legs folded. The assessment of this posture showed that the upper arm, lower arm, and wrist scores were 2, 2, and 3, respectively. Adding muscle score one and load score 1, the total wrist and arm score was 5. For the neck and trunk score, each section scored 3, and the leg score was 1. This score, added to section B's muscle and load score, became 7. It can be interpreted from the RULA sheet that this posture needs further investigation and that the change must be implemented for a better posture. In addition, workers were also strongly advised to change and improve their work posture immediately. Workers may not feel the impact of the work posture in a short time. However, the longer the work duration, the more workers' complaints, such as cramps, tingling, aches, and even back and neck pain, will gradually appear. The table below summarizes the RULA final score.

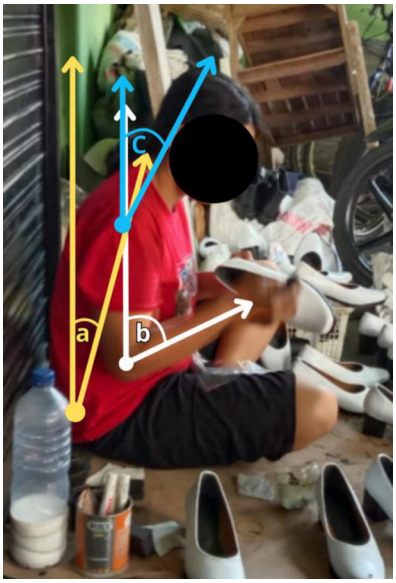


**Figure 5 Finishing**

**Table 2 Summarize the RULA Final Score**

| Worker in Section | Final Score |
|---|---|
| Process of cutting according to the pattern | 6 |
| Installation on a shoe mold | 7 |
| Gluing and pasting the sole | 6 |
| Outsole installation | 6 |
| Finishing | 7 |

Table 2 shows that the work postures observed resulted in final scores between 6 and 7.

## Discussion

Workers do their work by sitting on tiny chairs without backrests. Some even lie on the floor while doing their work. This work posture is not ergonomic and is uncomfortable for workers. The workplace is organized, and even some unused objects can still be seen around. Workers will have difficulty working, and sometimes work equipment may be difficult to find because many items are mixed with unused items.

The layout or design of the workplace will be primarily determined by the type of work and equipment used to carry out the work. While working conditions can be designed ergonomically by adjusting the work and equipment and workers who will use it.[6–8]

Workers' postures when cutting shoe materials according to the pattern were sitting, bent back, and head bowed (Figure 1). This posture puts extra pressure on the lower back and neck. The final RULA score for this posture was 6, meaning that further investigation and posture improvement are needed immediately, considering that this type of work posture looks like an extremely bent back.

If the potential ergonomic hazards are not immediately corrected, the workers will be at risk of experiencing health problems related to musculoskeletal. The risk of health problems and occupational diseases must be minimized so that workers are always healthy and safe when doing their jobs.[9]

Sitting work posture was also found among smoked fish workers in Demak. Workers sit in tiny chairs that make their backs bend and even twist

while working. As many as 87% of the smoking fish workers experienced musculoskeletal complaints, including 85% neck stiffness, 90% back pain, 95% leg tingling, and 75% pain in the shoulders and hands.[10]

Based on the measurement results using the RULA method, posture when installing a shoe mold (Figure 2) produces a score of 7, meaning that further investigation or research is needed to determine the impact that can arise from this posture. In addition, a change in work posture will likely be necessary given the very non-ergonomic work posture. Workers must work in a sitting posture for long periods and use their hands so that complaints of discomfort in the back, arms, and wrists will arise.[7,11]

A high prevalence of MSD symptoms was found among shoe workers in Iran. The average RULA score obtained is 6.3. It indicates that in most cases, workers' posture in the workplace needs to be investigated and changed immediately to prevent musculoskeletal injuries. The highest prevalence rates of MSDs were found in the shoulder, wrist, and arm areas. Based on statistical tests conducted show that several risk factors associated with upper extremity musculoskeletal disorders (UEMSD) symptoms include work experience, daily working hours, job satisfaction, work posture, work pressure, and discomfort at work, as well as individual factors such as age, gender, body mass index, working conditions, education level, and lack of regular physical/sports activity.[12]

A study on welding workers also found that non-ergonomic work postures such as sitting very low or squatting with the head lowered will cause complaints in several parts of the worker's body. As many as 100% of workers experience pain in the back, waist, and calves; 80% of workers experience neck pain; 70% of workers experience shoulder pain; and 10% of workers complain of arm pain.[13]

The measurement results using the RULA method show that the working posture when gluing and pasting the sole (Figure 3) is categorized at a score of 6. This indicates that further investigation is needed regarding the posture, which must be corrected immediately. It can be seen in the picture that workers are very uncomfortable in their sitting conditions; workers have to fold their legs for a long time. Of course, this will most likely cause complaints such as aches, cramps, and tingling. If the sitting posture is not immediately corrected, workers also tend to experience fatigue quickly.

This is in line with the results of a study in the shoe industry in Medan, which found that the activities carried out by workers are categorized as requiring further action. Activities carried out by workers do not make workers feel too much pain. However, an improvement in the working position and adequate rest are needed to restore the worker's condition and stamina after working with that posture.[14]

Research conducted on farmers in Iran showed that the highest prevalence of MSD symptoms was associated with the lower back (59.3%), knees (36.9%), and upper back (36.6%). The quick exposure check (QEC) scores indicated high or very high yields (action levels 3 and 4) in 83.1% of farmers. In addition, the checklist for ergonomic working conditions revealed that the workers' "work posture" index had the lowest average. It indicates poor ergonomic conditions, while "work equipment" has the highest average, indicating proper ergonomic conditions. In this study, it is also recommended that hazardous work postures be eliminated and that working conditions be improved.[15]

The work posture when the worker installed the outsole in Figure 4 is categorized at a score of 6 based on the RULA assessment. Further investigation is needed in this case, and it is necessary to improve the work posture to make it more ergonomic. It can be seen in the picture that workers do their work by sitting on the floor without chairs or cushions. This work posture will make workers feel very uncomfortable and will trigger complaints or health problems, such as knee pain, back and neck pain, and tend to get tired quickly.[16]

A similar posture was also found in a study in another shoe industry. Workers in these industries even look less effective due to poor working positions, so the worker postures become awkward, and work postures are static. Workers carry out their work in a sitting position continuously without using a sitting mat, with their necks bent to reach the work object with a bent back.[14] Studies on smoked fish workers found that when the sitting position was not comfortable/not ergonomic or sitting for too long, it caused backache or pain.[16]

A study examining the relationship between

MSD complaints and fatigue in nurses showed that ankles/legs, lower back, knees, and shoulders had the highest prevalence of work-related musculoskeletal symptoms (WMS) among nurses in the last 12 months before the study. Independent variables, including age, years of service, sex, smoking habits, shift work, and type of work, were significantly associated with WMS in different body regions, with odds ratios (OR) ranging from 1.635 to 2.835. In addition, WMS in several areas of the body is also associated with work fatigue. In this study, it is also recommended that ergonomic and organizational interventions be implemented to adjust the work of nurses, taking into account demographic/occupational characteristics to improve the health of the musculoskeletal system and relieve fatigue.[17]

Research conducted on workers in the manufacturing industry shows that workers believe that the pain/pain they feel is due to poor workstation design. The workers also suggested improvements to the workplace design to reduce pain complaints.[18]

In the finishing process (Figure 5), a bent posture was found because the worker was sitting on the floor with his legs folded. Based on the RULA measurement, this posture was included in the score category 7. Further investigation is needed about this work posture. In addition, workers are strongly advised to change and improve their work posture immediately. Workers may not feel the impact of the work posture in a short time. However, the longer the work duration, the more workers' complaints, such as cramps, tingling, aches, and even back and neck pain, will gradually appear.

A study of workers in a shoe factory in Iran found that the prevalence and severity of health complaints were very high in the study population. The mean RULA value of 6.2 indicates that, in most cases, workers' workplace posture needs to be investigated or investigated further. Some changes are even required as soon as possible. This study also showed that work experience, daily working hours, duration of continuous work without rest, feeling of pressure due to work, and work posture were significantly associated with musculoskeletal symptoms from various body areas. These findings help better understand the working conditions of jobs with similar work postures and activities and highlight the potential for ergonomic interventions to reduce musculoskeletal symptoms in this group of workers.[19]

Research on nurses shows that poor ergonomics, including work posture, caused WMSDs in addition to other factors, namely old age, length of work, high workload, work habits, high levels of physical activity, equipment availability, stress, and anxiety.[20] Other research findings also emphasize that any intervention program in the shoe industry or similar should focus on improving working conditions, primarily by designing ergonomically oriented workstations and hand tools.[14] It is necessary to improve work attitudes and ergonomics. Workplace stretching can be added to reduce musculoskeletal complaints and fatigue and increase productivity.[21]

Overcoming musculoskeletal risk factors through ergonomic interventions regarding workspace design, sitting/standing posture of workers, chairs, and hand postures during work and work-rest cycles can be realized with the cooperation of policymakers, companies, and workers.[21]

Overall, the work posture of workers in the leather shoe industry is in category 6–7. This means that the posture requires further investigation regarding the impact that will occur. In addition, it is also necessary to improve work posture as soon as possible so that the negative impact of non-ergonomic work postures can be avoided.

This research is limited to describing the level of work posture risk experienced by workers in the shoe industry in Semarang. It does not include a description of workers' musculoskeletal complaints. The author suggests further research on reducing or eliminating non-ergonomic work postures in the leather shoe industry and on workers' musculoskeletal complaints.

## Conclusions

It can be concluded that the workers in leather the leather industry's RULA score indicated that it's reinvestigation and needs a better posture. In addition, workers were also advised to change and improve their work posture immediately.

## Conflict of Interest

All authors convey that there is no conflict of

interest to all parties.

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## References

1. International Labour Organization. ILO supports inclusion of musculoskeletal disorders into the list of occupational diseases in China [Internet]. Geneva: International Labour Organization; 2022 [cited 2023 August 23]. Available from: https://www.ilo.org/resource/news/ilo-supports-inclusion-musculoskeletal-disorders-list-occupational-diseases.
2. Suryadi I, Rachmawati S. Work posture relations with low back pain complaint on partners part of PT "X" manufacture of tobacco products. J Vocational Health Stud. 2020;3(3):126–30.
3. Ekechukwu END, Useh E, Nna OL, Ekechukwu NI, Obi ON, Aguwa EN, et al. Ergonomic assessment of work-related musculoskeletal disorder and its determinants among commercial mini bus drivers and driver assistants (mini bus conductors) in Nigeria. PLoS One. 2021;16(12):e0260211.
4. European Agency for Safety and Health at Work. Musculoskelatal disorders [Internet]. Bilbao: European Agency for Safety and Health at Work; 2019 [cited 2023 September 13]. Available from: https://osha.europa.eu/en/themes/musculoskeletal-disorders.
5. Gómez-Galán M, Callejón-Ferre ÁJ, Pérez-Alonso J, Díaz-Pérez M, Carrillo-Castrillo JA. Musculoskeletal risks: RULA bibliometric review. Int J Environ Res Public Health. 2020;17(12):4354.
6. Dzikrillah N, Yuliani ENS. Analisis postur kerja menggunakan metode rapid upper limb assessment (RULA) studi kasus PT. TJ Forge Indonesia. J Ilm Tek Ind. 2015;3(3):150–5.
7. Hoe VC, Urquhart DM, Kelsall HL, Zamri EN, Sim MR. Ergonomic interventions for preventing work-related musculoskeletal disorders of the upper limb and neck among office workers. Cochrane Database Syst Rev. 2018;10(10):CD008570.
8. Jirapongsuwan A, Klainin-Yobas P, Songkham W, Somboon S, Pumsopa N, Bhatarasakoon P. The effectiveness of ergonomic intervention for preventing work-related musculoskeletal disorders in agricultural workers: a systematic review protocol. PLoS One. 2023;18(7):e0288131.
9. Ramadhani N, Rini WNE. Kajian identifikasi bahaya, analisis risiko dan pengendalian bahaya di PT X tahun 2021. An Nadaa J Kesehat Masy. 2021;8(2):168–79.
10. Setyaningsih Y, Wahyuni I, Ekawati. Identification of musculoskeletal disorder complaint, dermatitis incident and respiratory disorder in smoked fish worker. E3S Web Conf. 2020;202:12003.
11. Siddiqui LA, Banerjee A, Chokhandre P, Unisa S. Prevalence and predictors of musculoskeletal disorders (MSDs) among weavers of Varanasi, India: a cross-sectional study. Clin Epidemiol Glob Health. 2021;12:100918.
12. Veisi H, Choobineh A, Ghaem H, Faraji Kujerdi M, Barazandeh R, Barazandeh H. Upper extremity musculoskeletal symptoms among Iranian hand-woven shoe workers. Work. 2020;67(1):129–39.
13. El-Matury HJ. Evaluation of work station and working posture on welding section review of ergonomic factors in metal SME road court Medan. JIKA. 2020;5(2):235–47.
14. Nasution RH. Evaluasi postur kerja pada UMKM sepatu dengan metode rapid entire body assessment. JSR. 2020;2(1):72–5.
15. Momeni Z, Choobineh A, Razeghi M, Ghaem H, Azadian F, Daneshmandi H. Work-related musculoskeletal symptoms among agricultural workers: a cross-sectional study in Iran. J Agromedicine. 2020;25(3):339–48.
16. Ekawati E, Setyaningsih Y, Wahyuni I. Occupational safety and health hazards among smoked fish workers in Demak. GMHC. 2022;10(3):212–7.
17. Hosseini E, Daneshmandi H, Bashiri A, Sharifian R. Work-related musculoskeletal symptoms among Iranian nurses and their relationship with fatigue: a cross-sectional study. BMC Musculoskelet Disord.

2021;22(1):629.
18. Chiasson MÈ, Imbeau D, Major J, Aubry K, Delisle A. Influence of musculoskeletal pain on workers' ergonomic risk-factor assessments. Appl Ergon. 2015;49:1–7.
19. Dianat I, Salimi A. Working conditions of Iranian hand-sewn shoe workers and associations with musculoskeletal symptoms. Ergonomics. 2014;57(4):602-11.
20. Almhdawi KA, Alrabbaie H, Kanaan SF, Oteir AO, Jaber AF, Ismael NT, et al. Predictors and prevalence of lower quadrant work-related musculoskeletal disorders among hospital-based nurses: a cross-sectional study. J Back Musculoskelet Rehabil. 2020;33(6):885–96.
21. Suwartini NL, Tirtayasa K, Adiputra LMISH. The improvement of working posture and ergonomic workplace stretching decreased musculoskeletal complaint and fatigue and increased productivity of nurses. J Ergon Indones. 2020;6(2):105–12.
22. Hossain MD, Aftab A, Al Imam MH, Mahmud I, Chowdhury IA, Kabir RI, et al. Prevalence of work related musculoskeletal disorders (WMSDs) and ergonomic risk assessment among readymade garment workers of Bangladesh: a cross sectional study. PLoS One. 2018;13(7):e0200122.

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**RESEARCH ARTICLE**

# Effect of *Ketapang* Leaf Extract on HDL Levels of *Rattus norvegicus* Induced by High-fat Foods

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**Abstract**

High-density lipoprotein (HDL) functions to transport cholesterol from the blood vessel endothelium to the liver and excreted through the digestive tract so that it does not cause dyslipidemia, which is a risk factor for cardiovascular disease. Current treatment for dyslipidemia uses long-term statin drugs, which can cause various side effects, so herbal alternatives such as *ketapang* leaves (*Terminalia catappa* L.), which contain flavonoids and have the effect of increasing HDL levels, needed to be evaluated to achieve that reason. This study aims to evaluate whether *ketapang* leaf extract has an effect on increasing blood HDL levels. This research used experimental animals with a pretest and a posttest with a control group design. It was conducted at the Biochemistry Laboratory and Laboratory Animal Management Unit, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan, from September to December 2023. This study used Wistar strain male white rats (*Rattus norvegicus*) that were induced on a high-fat diet, divided into five groups: negative control, positive control (given simvastatin), treatment 1 (given 21 mg/kgBW *ketapang* leaf extract), treatment 2 (given 42 mg/kgBW *ketapang* leaf extract) and treatment 3 (given 84 mg/kgBW *ketapang* leaf extract). The study results showed a significant difference ($p<0.05$) in the average HDL levels before and after treatment in the positive control, treatment 2, and therapy three groups. Thus, the study concluded that *ketapang* leaf extract affected increasing HDL levels of male white rats (*Rattus norvegicus*).

**Keywords:** HDL, high-fat diet, *ketapang* leaf extract, *Rattus norvegicus*

## Introduction

Cholesterol is vital in cell growth, produces sex hormones and vitamin D, and controls nerve and brain functions. However, excess cholesterol in the blood can cause dyslipidemia and increase the risk of cardiovascular disease. Cholesterol cannot dissolve in the blood, so lipoproteins are needed to distribute cholesterol throughout the body.[1] Dyslipidemia is a condition of abnormal lipid levels in the blood circulation, decreased levels of high-density lipoprotein (HDL), increased levels of cholesterol, low-density lipoprotein (LDL), and triglyceride levels.[2] One type of cholesterol that has a good role for the body is HDL, which is responsible for transporting cholesterol from the blood vessel endothelium so that it does not accumulate cholesterol in the blood vessel endothelium, which is then taken to the liver and excreted through the digestive tract.[3] Increasing blood cholesterol levels will impact the risk of atherosclerosis; the presence of HDL will clean blood vessels from cholesterol. Besides transporting cholesterol, HDL also widens blood vessels due to increased nitric oxide production.[4] There were no symptoms associated with low HDL levels, but they did increase the risk of cardiovascular disease.[5] Cardiovascular disease accounts for 30% of all deaths in the world; this is due to uncontrolled and untreated dyslipidemia.[6,7]

Current treatment for dyslipidemia uses statin drugs. One group of commonly used statins is simvastatin. Simvastatin works by inhibiting the HMG-CoA enzyme, which plays a role in cholesterol synthesis in the liver, so that cholesterol production is reduced.[8] Although it is beneficial for lowering cholesterol levels, there are side effects due to the use of this class of statin

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drugs, such as obstipation, nausea, headaches, stuffy nose, sneezing, and sore throat; in some people, it can cause forgetfulness or confusion. In addition, drug interaction effects can occur if used with certain drugs, such as increasing the risk of muscle disorders (myopathy), rhabdomyolysis, the risk of bleeding, and impaired liver and kidney function.[9,10] So, alternative medicine is needed for treatment, such as herbal treatment, which is expected to minimize side effects and provide good benefits for the body.

*Ketapang* (*Terminalia catappa* L.) is known as a tropical almond belonging to the family Combretaceae. They are grown mainly for shade and ornamental purposes and are primarily found in yards. The research results show that *ketapang* leaves contain flavonoids, saponins, and tannins. Flavonoids are one of the polyphenolic compounds that have antihypercholesterolemic effects; polyphenols can inhibit the work of HMG-CoA reductase so that cholesterol synthesis in the body decreases, and flavonoids can also increase HDL levels by increasing apolipoprotein one, which is the essential ingredient for forming HDL.[11,12]

Previous research shows that *ketapang* leaves have a role in reducing total cholesterol levels. It is known that the highest average reduction in cholesterol levels in male mice (*Mus musculus* L.) was found in *ketapang* leaf extract at 8 mg/kg body weight. The group given 16 mg *ketapang* leaf extract per kg body weight showed almost the same results as the positive control group (simvastatin).[13] This study aims to evaluate whether *ketapang* leaf extract has an effect on increasing blood HDL levels of male Wistar white rats (*Rattus norvegicus*) induced by a high-fat diet.

## Methods

The research used an experimental study method using experimental animals with a pretest and a posttest with a control group design. The research was conducted at the Biochemistry Laboratory and Laboratory Animal Management Unit, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Medan, from September to December 2023. The population of this study was adult male Wistar white rats (*Rattus norvegicus*) aged >3 months, weighing 100–150 grams, healthy and active, and had never been used in research. In this study, the sample was divided into five groups, and the number of samples per group was calculated using the Federer formula; the number of samples per group was five rats. The hypercholesterolemic rats were created by giving them a high-fat diet using quail egg yolks.

The research was carried out for twenty-seven days. The rats acclimatized for seven days to adapt to the environment. On the eighth day, they were separated into groups and given egg yolk for eight days, and on the fourteenth day, the LDL levels were checked before treatment; on the fifteenth to the twenty-fifth day they were given *ketapang* leaf extract with various doses, namely group P1 was given 21 mg/kgBW, P2 was given 42 mg/kgBW and P3 was given 84 mg/kgBW, on the twenty-sixth day, HDL levels were checked after treatment (Figure). This research was registered with the Health Research Ethics Committee, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, with registration number 1097/KEPK/FKUMSU/2023. Statistical analysis was done using SPSS, normality, and homogeneity tests before comparing the differences between groups using one-way ANOVA followed by post hoc LSD.

## Results

The average HDL levels of male Wistar white rats after being given egg yolk for 7 days and before being given *ketapang* leaf extract were as follows: negative control group was 33 mg/dl, positive control group was 27.20 mg/dl, treatment group 1 was 27.60 mg/dl, treatment group 2 was 25.80 mg/dl and treatment group 3 was 25 mg/dl. The average HDL levels of male Wistar white rats after being given *ketapang* leaf extract were as follows: negative control was 34.40 mg/dl, positive control was 40.80 mg/dl, treatment group 1 was 30 mg/dl, treatment group 2 was 32 mg/dl and treatment group 3 was 39.20 mg/dl.

In Table 1, it can be seen that there is a significant difference in the average HDL before and after treatment ($p<0.05$) in the positive control group; there is an increase in the average HDL level after administration of simvastatin. Likewise, the average HDL before and after treatment in groups K2 and K3 increased the average HDL levels after administering *ketapang* leaf extract.

Table 2 shows no significant difference

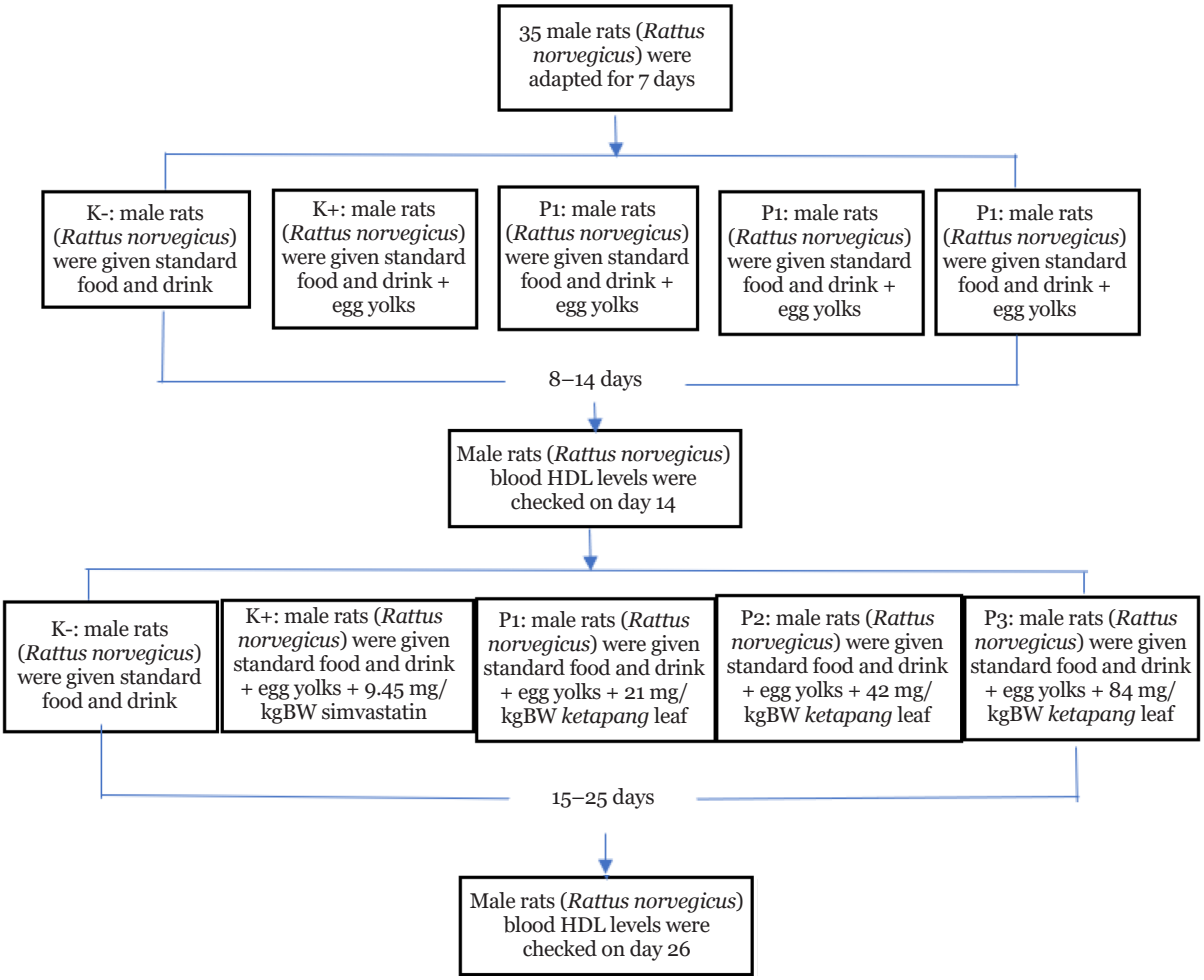


**Figure Research Flow**

(p>0.05) between the average HDL levels in the K+ and P3 groups, so the dose of *ketapang* leaf extract given to the P3 group can be taken as an effective dose.

## Discussion

In this study, all groups of male Wistar white rats, except the negative control group, were given high-fat food in quail egg yolks to create a hypercholesterolemia model of rats. Quail eggs have the second highest cholesterol content after duck eggs and have twice as high cholesterol levels (844 mg/dl) as chicken eggs (423 mg/dl).[14,15] After seven days of giving rats high-fat food, there was a decrease in the average HDL blood levels in all

**Table 1 Difference in Average HDL Levels before and after Treatment**

| Groups | Before Treatment Mean±SD (mg/dl) | After Treatment Mean±SD (mg/dl) | p |
|---|---|---|---|
| Negative control (K-) | 33.00±4.06 | 34.40±2.96 | 0.500 |
| Positive control (K+) | 27.20±2.77 | 40.80±1.92 | 0.000 |
| Treatment 1 (P1) | 27.60±3.04 | 30.00±3.87 | 0.250 |
| Treatment 2 (P2) | 25.80±4.49 | 32.00±2.54 | 0.004 |
| Treatment 3 (P3) | 25.00±3.67 | 39.20±2.14 | 0.000 |

**Table 2 Comparison of Average HDL Levels between Treatment Groups**

| | K- | K+ | P1 | P2 | P3 |
|---|---|---|---|---|---|
| K- | | | | | |
| K+ | 0.003 | | | | |
| P1 | 0.039 | <0.001 | | | |
| P2 | 0.250 | <0.001 | 0.337 | | |
| P3 | 0.025 | 0.441* | <0.001 | 0.001 | |

Note: LSD post hoc test, *effective dose

groups compared to the negative control group. High-fat foods cause a decrease in HDL levels by increasing lipid absorption so that the amount of lipid in peripheral cells will increase, followed by an increase in reverse cholesterol transport activity and the inability to compensate for and decrease HDL levels.[16] Hypercholesterolemia causes a decrease in HDL levels because cholesterol tends to affect the balance of total cholesterol in the blood, which can affect the level of HDL.[17,18] Hypercholesterol can also change the activity of the lipoprotein lipase enzyme, which plays a role in the formation and metabolism of HDL, thereby causing decreased production or increased degradation of HDL.[16]

In this study, simvastatin was used as a positive control because simvastatin is the most effective drug that lowers cholesterol levels. It works by inhibiting cholesterol synthesis in the liver by inhibiting the HMG-CoA reductase enzyme.[19,20]

The study showed the effect of giving *ketapang* leaf extracton the HDL levels of male white Wistar rats induced by a high-fat diet. There was an increase in HDL levels in each treatment group after being given *ketapang* leaf extract. The average blood HDL levels of white male Wistar rats in the treatment groups with doses of 21 mg/kgBW, 42 mg/kgBW, and 84 mg/kgBW after being given *ketapang* leaf extract increased compared to the average HDL levels before being given it. This is in line with previous research, which shows that *ketapang* leaves affect the increase in total cholesterol and LDL levels and decrease HDL levels.[13]

Based on the results of phytochemical tests, the secondary metabolite chemical compound group in *ketapang* leaf simplex contains flavonoids, tannins, saponins, and alkaloids.[21] Flavonoids inhibit cholesterol synthesis, cholesterol esterification synthesis, and inhibit HMG-CoA reductase activity.[22] Flavonoids show the potential to improve HDL function through their well-documented effects on cellular antioxidant status and inflammation.[12] Following previous research, organic celery extract containing flavonoids increases HDL levels in white mice fed high-fat diets.[23] Flavonoid compounds also have a mechanism for increasing HDL levels by increasing the release of cholesterol from macrophages, increasing the expression of ATP-binding cassette, and increasing apolipoprotein A1, the primary material for the formation of HDL.[24] Tannin extract can reduce the accumulation of cholesterol in the blood, accelerate the removal of cholesterol through feces, and does not occur through the enterohepatic cycle.[25] Saponin was able to inhibit dietary fat absorption by inhibiting pancreatic lipase activity, its ability to prevent hypercholesterolemia, and increase HDL.[26] Alkaloids have been demonstrated to regulate lipid metabolism by enhancing energy metabolism, promoting lipid phagocytosis, and inhibiting adipocyte proliferation and differentiation.[27]

The effectiveness of *ketapang* leaf extract in increasing HDL levels can be seen statistically in the LSD post hoc test (Table 2). The HDL levels of the group given *ketapang* leaf extract with various concentrations (P1, P2, P3) were compared with the positive control group, namely the group given simvastatin. The HDL levels of groups P1 and P2 showed significant differences with the positive control group, while group P3 did not show significant differences with the control group. This indicates that 84 mg/kgBW *ketapang* leaf extract has the same effect as simvastatin.

## Conclusion

This research concludes that *ketapang* leaf extract could increase the HDL levels of male white Wistar rats (*Rattus norvegicus*) induced by a high-fat diet.

## Conflict of Interest

The authors declared no conflict of interest.

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## References

1. Corallo R. Cholesterol. In: Tugman B, Baxter S, Wu Y, editors. A guy's guide: what every man needs to know about their health [e-book]. Montreal: Pressbooks; 2021 [cited 2024 June 12]. Available from: https://pressbooks.pub/btugman2021/chapter/cholesterol.
2. Aswania GM, Yasmin AAADA. Dislipidemia sebagai prediktor kejadian kardiovaskular mayor pada pasien infark miokard akut. EJ Medika Udayana. 2020;9(11):91–100.
3. Apriyanto DR, Frisqila C. Perbandingan efektivitas ekstrak dan fermentasi buah naga merah terhadap penurunan kadar kolesterol low density lipoprotein (LDL) pada tikus putih yang dibuat hiperkolesterolemia. Tunas Medika J Kedokteran Kesehat. 2016;3(3):1–5.
4. Rafsanjani MS, Asriati A, Kholidha AN, Alifariki LO. Hubungan kadar high density lipoprotein (HDL) dengan kejadian hipertensi. J Prof Medika. 2019;13(2):74–81.
5. Zhao X, Wang D, Qin L. Lipid profile and prognosis in patients with coronary heart disease: a meta-analysis of prospective cohort studies. BMC Cardiovasc Disord. 2021;21(1):69.
6. Braun A. Does low HDL cholesterol cause symptoms? [Internet]. New York: Verywell Health; 2024 [cited 2024 July 10]. Available from: https://www.verywellhealth.com/low-hdl-symptoms-signs-symptoms-and-complications-5188643.
7. Pirillo A, Casula M, Olmastroni E, Norata GD, Catapano AL. Global epidemiology of dyslipidaemias. Nat Rev Cardiol. 2021;18(10):689–700.
8. Ramkumar S, Raghunath A, Raghunath S. Statin therapy: review of safety and potential side effects. Acta Cardiol Sin. 2016;32(6):631–9.
9. Abu Mellal A, Hussain N, Said AS. The clinical significance of statins-macrolides interaction: comprehensive review of in vivo studies, case reports, and population studies. Ther Clin Risk Manag. 2019;15:921–36.
10. Valentovic M. Simvastatin. In: Enna SJ, Bylund DB, editors. xPharm: the comprehensive pharmacology reference. Philadelphia: Elsevier; 2007. p. 1–4.
11. Zeka K, Ruparelia K, Arroo RRJ, Budriesi R, Micucci M. Flavonoids and their metabolites: prevention in cardiovascular diseases and diabetes. Diseases. 2017;5(3):19.
12. Millar CL, Duclos Q, Blesso CN. Effects of dietary flavonoids on reverse cholesterol transport, HDL metabolism, and HDL function. Adv Nutr. 2017;8(2):226–39.
13. Maharadingga M, Pahriyani A, Arista D. Uji aktivitas ekstrak etanol 70% daun ketapang (*Terminalia catappa* L.) pada hamster Syrian jantan hiperglikemia dan hiperkolesterolemia dengan parameter pengukuran kolesterol total dan LDL. Lumbung Farmasi. 2021;2(2):80–8.
14. Waluyo J, Wahyuni D. The effect of ketapang leaf extracts (*Terminalia catappa* L.) on the cholesterol levels of male mice (*Mus musculus* L.) hypercholesterolemia. IJAERS. 2017;4(7):45–9.
15. Thomas KS, Jagatheesan PNR, Reetha TL, Rajendran D. Nutrient composition of Japanese quail eggs. IJSET. 2016;5(3):1293–5.
16. Moffatt RJ, Stamford B. Lipid metabolism and health. Boca Raton: CRC Press; 2006.
17. Nurfianti A, Tribudi YA. Kadar malondialdehid (MDA) dan kolesterol pada telur puyuh yang diberi pakan tambahan tepung pegagan (*Centella asiatica*). JTP. 2016;17(3):187–94.
18. Guo J, Chen S, Zhang Y, Liu J, Jiang L, Hu L, et al. Cholesterol metabolism: physiological regulation and diseases. MedComm. 2024;5(2):e476.
19. Pappan N, Awosika AO, Rehman A. Dyslipidemia. [Updated 2024 Mar 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 July 16]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560891.
20. Feingold KR. Cholesterol lowering drugs. [Updated 2024 Feb 12]. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.

com, Inc.; 2000 [cited 2024 July 20]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK395573.
21. Herli MA, Wardaniati I. Skrining fitokimia ekstrak etanol dan fraksi daun ketapang yang tumbuh di sekitar Univ. Abdurrab, Pekanbaru. JOPS. 2019;2(2):38–42.
22. Baskaran G, Salvamani S, Ahmad SA, Shaharuddin NA, Pattiram PD, Shukor MY. HMG-CoA reductase inhibitory activity and phytocomponent investigation of *Basella alba* leaf extract as a treatment for hypercholesterolemia. Drug Des Devel Ther. 2015;9:509–17.
23. Syafitri S, Ayu PR, Wijaya SM. Pengaruh pemberian ekstrak seledri (*Apium graveolens* L.) organik terhadap kadar high density lipoprotein (HDL) tikus putih (*Rattus novergicus*) galur Sprague Dawley yang diberi pakan tinggi lemak. J Kesehat Tambusai. 2022;3(1):88–95.
24. Duan Y, Gong K, Xu S, Zhang F, Meng X, Han J. Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics. Signal Transduct Target Ther. 2022;7(1):265.
25. Yao J, Chen P, Apraku A, Zhang G, Huang Z, Hua X. Hydrolysable tannin supplementation alters digestibility and utilization of dietary protein, lipid, and carbohydrate in grass carp (*Ctenopharyngodon idellus*). Front Nutr. 2019;6:183.
26. Marrelli M, Conforti F, Araniti F, Statti GA. Effects of saponins on lipid metabolism: a review of potential health benefits in the treatment of obesity. Molecules. 2016; 21(10):1404.
27. Ma Z, Wang S, Miao W, Zhang Z, Yu L, Liu S, et al. The roles of natural alkaloids and polyphenols in lipid metabolism: therapeutic implications and potential targets in metabolic diseases. Curr Med Chem. 2023;30(32):3649–67.

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**RESEARCH ARTICLE**

# COVID-19 Treatment Patterns in Patients with Acute Respiratory Failure at Dr. Hasan Sadikin General Hospital Bandung in 2021–2022

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**Abstract**

Acute respiratory failure is the most common complication and cause of death in COVID-19 patients. The COVID-19 medication has yet to be discovered. COVID-19 treatment guidelines are constantly being updated. This study aims to determine the COVID-19 treatment patterns in patients with acute respiratory failure at Dr. Hasan Sadikin General Hospital Bandung in 2021–2022. This retrospective, descriptive study used systematic random sampling to examine medical records of COVID-19 patients with acute respiratory failure at Dr. Hasan Sadikin Central General Hospital between June 2021 and June 2022. Gender, age, length of stay, outcome, comorbidities, and pharmacological and non-pharmacological treatment data were analyzed by SPSS software. This study included 120 COVID-19 patients with acute respiratory failure, with the majority of patients are male (55.83%), 30–60 years old (55.83%), length of stay of 1–3 days (52.5%), and have disease severity at severe condition (43.33%) and one comorbidity (37.5%). Patients mostly received non-rebreathing oxygen mask (54.2%), antiviral remdesivir (83.3%), corticosteroid dexamethasone (76.7%), enoxaparin anticoagulants (61.7%), a combination of vitamin C, vitamin D, and multivitamins (45.8%), and two antibiotics (33.3%). Additional treatments include tocilizumab (0.8%), intravenous immunoglobulin (2.5%), and convalescent plasma (0.8%). Statistical analysis shows that patients who stay at the hospital longer, have less or no comorbidities, and are given oxygen therapy have a significant possibility of recovering. Treatments commonly prescribed to COVID-19 patients with acute respiratory failure are antivirals, corticosteroids, anticoagulants, vitamins, and antibiotics, while the administration of oxygen therapy has a significant probability of recovery.

**Keywords:** Acute respiratory failure, COVID-19, pattern, treatment

## Introduction

Coronavirus disease 2019 (COVID-19) is a worldwide emergency due to its rapid spread and high mortality rate.[1] COVID-19 is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[2] The World Health Organization (WHO) declared COVID-19 a global pandemic on 11 March 2020.[3]

The primary target of the coronavirus is the human respiratory system. Patients with COVID-19 may exhibit a range of symptoms, from asymptomatic to critical, followed by acute respiratory failure.[4] Acute respiratory failure is the most prevalent complication and a leading cause of death among COVID-19 patients. For COVID-19 patients with acute respiratory failure, the mortality rate reaches 93%.[5] Patients with COVID-19 who develop acute respiratory failure had a lower quality of life compared to those who are not infected.[6]

Existing research on the management of COVID-19 with acute respiratory failure is still limited. The COVID-19 therapy guidelines are still being revised with the most recent scientific findings. Inappropriate drug administration in COVID-19 patients with acute respiratory failure can result in adverse drug reactions, increase mortality, and decrease the cost-effectiveness of treatment.[7] This study aims to identify the COVID-19 treatment patterns in patients with acute respiratory failure at Dr. Hasan Sadikin Central General Hospital Bandung in 2021–2022.

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## Methods

This retrospective, descriptive study was conducted on the medical records of COVID-19 patients with acute respiratory failure at Dr. Hasan Sadikin Central General Hospital from October to November 2022, with prior approval from the Research Ethics Committee Universitas Padjadjaran, ethical exemption number 863/UN6.KEP/EC/2022.

The study's sample consisted of the entire population that met the inclusion criteria: patients diagnosed with a critical degree of COVID-19 and acute respiratory failure between June 2021 and June 2022. The total required sample size was 120, and the samples were selected using systematic random sampling (Figure 1). Medical records with insufficient information were excluded.

Data extracted from the medical record included gender, age (the patient's age recorded in the medical record when he was first admitted to the hospital), length of stay (calculated from the date of admission to the date of discharge), outcome (the patient's condition when discharged from the hospital), comorbid diseases, and treatment (pharmacological and non-pharmacological) during at the hospital. The data was analyzed using both Microsoft® Excel 2021 and IBM® SPSS® 26th version.

## Results

Two hundred and seventeen of the 337 samples collected did not meet the inclusion criteria and were thus excluded from this study. A total of 120 patients' remaining data were analyzed. Table 1 showed that most patients in this study were male (55.83%) instead of female (44.17%). Most of the patients came from 30–60 years old (55.83%) and with disease severity at severe condition (43.33%). The median length of hospital stay was 2 days (range: 0–33 days), and most patients stayed there for 1–3 days (52.50%).

As shown in Table 1, in all variables, most patients died (90% of total patients of 120). Based on statistical analysis, variable length of stays showed a significant negative correlation with the outcome. It was indicated that patients who stay at the hospital longer have the probability

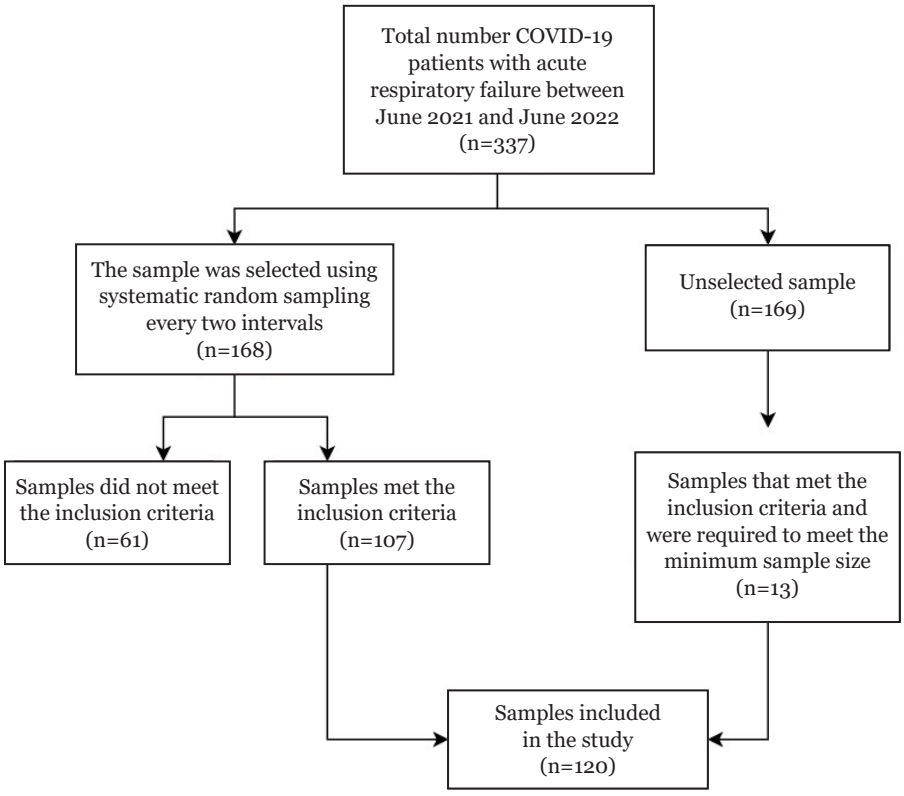


**Figure 1 Study Samples Selection**

**Table 1 Baseline Characteristic of the Study**

| Variables | Outcome (n=120) | | Total n (%) | Spearman Rho Test | |
|---|---|---|---|---|---|
| | Death n (%) | Recovered n (%) | | Correlation Coefficient | p |
| Gender | | | | −0.032 | 0.731 |
| Male | 58 (86.57) | 6 (11.32) | 67 (55.83) | | |
| Female | 47 (88.68) | 9 (13.43) | 53 (44.17) | | |
| Age groups (years) | | | | 0.041 | 0.655 |
| <30 | 3 (60.00) | 2 (40.00) | 5 (4.17) | | |
| 30–60 | 60 (89.55) | 7 (10.45) | 67 (55.83) | | |
| >60 | 42 (87.50) | 6 (12.50) | 48 (40.00) | | |
| Length of stay (days) | | | | −0.265 | 0.003* |
| <1 | 13 (92.86) | 1 (7.14) | 14 (11.67) | | |
| 1–3 | 60 (95.24) | 3 (4.76) | 63 (52.50) | | |
| >3 | 32 (74.42) | 11 (25.58) | 43 (35.83) | | |
| Disease severity | | | | 0.082 | 0.374 |
| Severe | 50 (84.75) | 9 (15.25) | 59 (49.17) | | |
| Critical | 55 (90.16) | 6 (9.84) | 61 (50.83) | | |

of recovering. A similar condition was shown at variable in gender that has a negative correlation with the outcome but not significantly. On the other hand, disease severity was shown to have a positive correlation but not significantly with the outcome, which means severe disease severity has the probability of recovery.

Most patients (37.5%) had at least one comorbid condition, as shown in Table 2. Some patients had two comorbid diseases (25.8%), three comorbid diseases (4.2%), and more than three comorbid diseases (7.5%). Most of the patients who died in this study had 1 or 3 comorbid diseases. Based on statistical analysis, a number of comorbid diseases have a significant positive correlation with the outcome, which means patients with fewer or no comorbidities have a probability of recovering. The table of comorbid diseases (Figure 2) showed that hypertension (27.7%) and diabetes mellitus (23.3%) are the two most prevalent comorbid diseases among patients in this study.

Table 3 shows the patient's treatment (pharmacological and non-pharmacological). All patients received oxygen therapy with the most frequently employed devices were non-rebreathing oxygen face masks (54.2%) and invasive mechanical ventilation (21.7%). Antivirals were administered to 107 patients (89.2%), with remdesivir (83%) being the most common. Corticosteroids were administered to 105 (87.5%) patients. The most frequently used corticosteroid was dexamethasone (76.7%). Anticoagulants were administered to 108 patients (90%), with enoxaparin, a low molecular weight heparin (61.7%), the most commonly administered type. Only one patient (0.8%) was administered tocilizumab, an anti-IL-6 agent. One

**Table 2 History of Comorbidities in COVID-19 Patients with Acute Respiratory Failure**

| Number of Comorbidities | Outcome (n=120) | | Total n (%) | Spearman Rho Test | |
|---|---|---|---|---|---|
| | Death n (%) | Recovered n (%) | | Correlation Coefficient | p |
| No comorbidities | 28 (23.3) | 2 (1.7) | 30 (25.0) | | |
| 1 comorbidity | 43 (35.8) | 2 (1.7) | 45 (37.5) | | |
| 2 comorbidities | 28 (23.3) | 3 (2.5) | 31 (25.8) | 0.261 | 0.004* |
| 3 comorbidities | 5 (4.2) | 0 (0.0) | 5 (4.2) | | |
| >3 comorbidities | 4 (3.3) | 5 (4.2) | 9 (7.5) | | |

Note: *significant difference at p<0.05

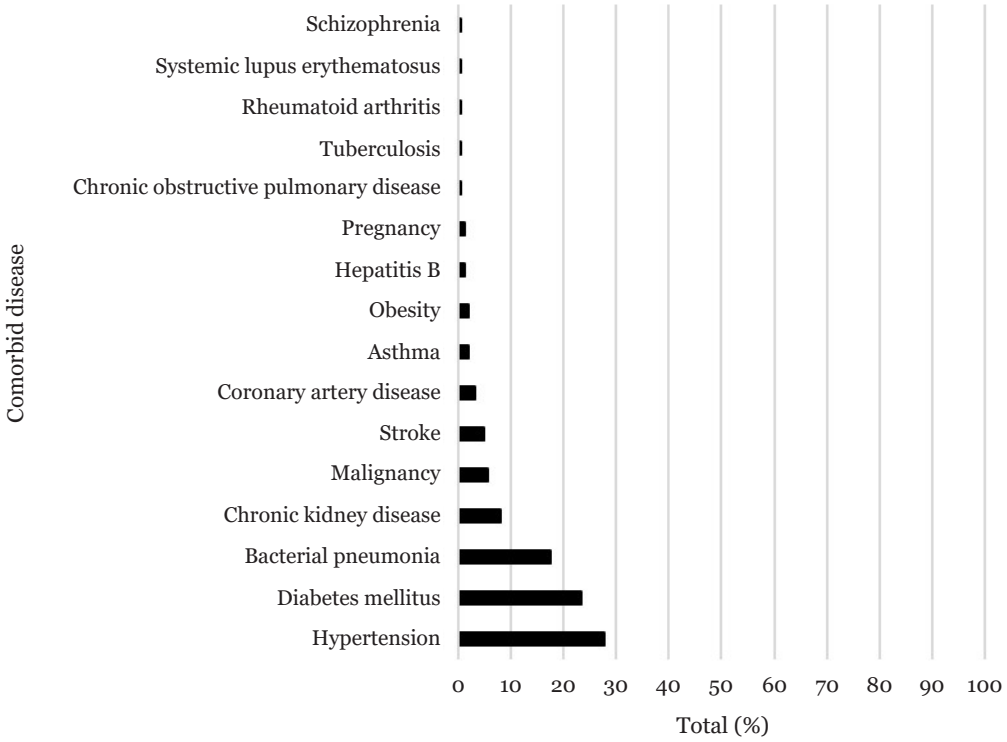


**Figure 2 Comorbidities in COVID-19 with Acute Respiratory Failure**

hundred and six of 120 patients (88.3%) received vitamins, with vitamin C, D, and multivitamins (45.8%) being the most common combination. Antibiotic therapy was administered to 68 patients (43.3%), and most (33.3%) were given two antibiotics.

Based on statistical analysis of the treatment of COVID-19, it was shown that oxygen therapy has a positive significant correlation with the outcome. It was indicated that the administration of oxygen therapy has the probability of recovery. A positive correlation, but not significant, with the outcome shown when administering antiviral, corticosteroid, and antibiotics to the patients means that administering these drugs has the possibility of recovery. On the other hand, a negative but insignificant correlation was shown with the administration of anticoagulants, anti-IL-6, vitamins, and supplements, which means the treatments didn't show a probability of recovery.

Table 4 shows the additional treatments administered to COVID-19 patients. Intravenous immunoglobulin (2.5%) and convalescent plasma (0.8%) were administered as adjunctive therapies to a total of 4 patients (3.3%). Based on statistical analysis, additional treatments have a negative correlation with the outcome, which means additional treatment didn't show a probability of recovery.

## Discussion

Between June 2021 and June 2022, there were 337 patients (10.6%) at Dr. Hasan Sadikin General Hospital Bandung were confirmed to have COVID-19 and acute respiratory failure. COVID-19 primarily targets the respiratory system and is characterized by an immune dysregulation that causes a "cytokine storm" in the body, particularly in the lung tissue.[8]

According to subject characteristics data, the mean age in this study was 54.9±13.6 years, with the majority falling within the 30–60 age group. But it also showed that younger age can recover. Several factors, including a decline in immune function, pre-existing health conditions, and the inflammatory response, significantly impact recovery from COVID-19 across different age groups. This is comparable to a study conducted by Xu et al.[9] in China, which found that the median age of patients was 56.5 years (range: 47.5–67.8),

**Table 3 Treatment of COVID-19 Patients with Acute Respiratory Failure**

| Treatment | Outcome (n=120) | | Total n (%) | Spearman Rho Test | |
|---|---|---|---|---|---|
| | Death n (%) | Recovered n (%) | | Correlation Coefficient | p |
| Oxygen therapy | | | | 0.298 | 0.001* |
| NRM | 63 (52.5) | 2 (1.7) | 65 (54.2) | | |
| IMV | 23 (19.2) | 3 (2.5) | 26 (21.7) | | |
| NRM+HFNC | 10 (8.3) | 2 (1.7) | 12 (10.0) | | |
| HFNC | 6 (5.0) | 2 (1.7) | 8 (6.7) | | |
| NRM+IMV | 3 (2.5) | 3 (2.5) | 6 (5.0) | | |
| NRM+HFNC+IMV | 3 (2.5) | 0 (0.0) | 3 (2.5) | | |
| Antiviral | | | | 0.130 | 0.157 |
| Remdesivir | 89 (74.2) | 11 (9.2) | 100 (83.3) | | |
| No antiviral | 13 (10.8) | 0 (0.0) | 13 (10.8) | | |
| Remdesivir+favipiravir | 4 (3.3) | 0 (0.0) | 4 (3.3) | | |
| Favipiravir | 2 (1.7) | 1 (0.8) | 3 (2.5) | | |
| Corticosteroid | | | | 0.132 | 0.149 |
| Dexamethasone | 82 (68.3) | 10 (8.3) | 92 (76.7) | | |
| No corticosteroid | 15 (12.5) | 0 (0.0) | 15 (12.5) | | |
| Dexamethasone+methylprednisolone | 9 (7.5) | 2 (1.7) | 11 (9.2) | | |
| Methylprednisolone | 1 (0.8) | 0 (0.0) | 1 (0.8) | | |
| Methylprednisolone+hydrocortisone | 1 (0.8) | 0 (0.0) | 1 (0.8) | | |
| Anticoagulant | | | | −0.147 | 0.108 |
| Enoxaparin | 70 (58.3) | 4 (3.3) | 74 (61.7) | | |
| UFH+enoxaparin | 19 (15.8) | 5 (4.2) | 24 (20.0) | | |
| No anticoagulant | 11 (9.2) | 1 (0.8) | 12 (10.0) | | |
| UFH | 8 (6.7) | 2 (1.7) | 10 (8.3) | | |
| Anti-IL-6 | | | | −0.035 | 0.707 |
| No anti-IL-6 | 107 (89.2) | 12 (10.0) | 119 (99.2) | | |
| Tocilizumab | 1 (0.8) | 0 (0.0) | 1 (0.8) | | |
| Vit and supplement | | | | −0.013 | 0.886 |
| Vit C+vit D+multivitamin | 47 (39.2) | 8 (6.7) | 55 (45.8) | | |
| Multivitamin | 15 (12.5) | 1 (0.8) | 16 (13.3) | | |
| Vit D+multivitamin | 13 (10.8) | 1 (0.8) | 14 (11.7) | | |
| No vit | 14 (11.7) | 0 (0.0) | 14 (11.7) | | |
| Vit C+vit D | 13 (10.8) | 0 (0.0) | 13 (10.8) | | |
| Vit C | 2 (1.7) | 0 (0.0) | 2 (1.7) | | |
| Multivitamin+vit K | 1 (0.8) | 1 (0.8) | 2 (1.7) | | |
| Vit C+vit D+vit K+multivitamin | 1 (0.8) | 1 (0.8) | 2 (1.7) | | |
| Vit C+multivitamin | 1 (0.8) | 0 (0.0) | 1 (0.8) | | |
| Vit B+vit C+multivitamin | 1 (0.8) | 0 (0.0) | 1 (0.8) | | |
| Antibiotic | | | | 0.074 | 0.423 |
| No antibiotics | 51 (42.5) | 1 (0.8) | 52 (43.3) | | |
| 2 antibiotics | 36 (30) | 4 (3.3) | 40 (33.3) | | |
| 3 antibiotics | 7 (5.8) | 2 (1.7) | 9 (7.5) | | |
| 4 antibiotics | 5 (4.2) | 3 (2.5) | 8 (6.7) | | |
| 1 antibiotic | 6 (5.0) | 1 (0.8) | 7 (5.8) | | |
| >4 antibiotics | 3 (2.5) | 1 (0.8) | 4 (3.3) | | |

Note: *significant difference at p<0.05, NRM: non-rebreathing oxygen mask, IMV: invasive mechanical ventilation, HFNC: high-flow nasal cannula, UFH: unfractionated heparin, vit: vitamin

and the majority were under 70 years.

On the other hand, based on gender, male patients outnumbered female patients. This finding is consistent with Bhatraju et al.,[10] indicating that COVID-19 with acute respiratory failure is more prevalent in males. Biological

(different immune responses in males and females) and social factors, such as a high-risk lifestyle, may contribute to the high incidence and severity of COVID-19 infection in men.[11]

The median length of stay of patients was 2 days (range: 0–33 days), in contrast to the results of the study by COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators, which showed that the median length of stay of patients was 23 days (range: 13–39 days).[12] Most of the patients in this study died (90%). When this study was conducted in 2021–2022, Indonesia experienced two peaks of COVID-19 due to the Delta and Omicron variants. Compared to previous variants, the Delta was linked with a greater risk of death. A high number of hospitalizations, a shortage of hospital beds, a lack of ICU spaces, and a lack of medical supplies were all factors that could have contributed to this period's high fatality rate and short hospital length of stay.[13]

However, based on statistical analysis, patients who stay longer in hospital have the possibility of recovering. This study is in line with the Faes et al.[14] study, which observed that in elderly patients, the median length of stay for patients who die varies between 5.7 days and 15.7 days, while for patients who recover, it varies between 15.7 days. It may be related to improved clinical experience and treatments throughout the epidemic.

The majority of patients have a comorbid disease. This study is in line with Guan et al.,[15] which states more than half of patients (52.56%) who died due to COVID-19 had more than two comorbidities, and the rest had only one comorbid. Hypertension, diabetes, and bacterial pneumonia are the most prevalent coexisting conditions. Hypertension and diabetes were the most common comorbidities in patients' deaths due to COVID-19. According to Djaharuddin et al.[16] shows that the most comorbidities were hypertension (42.31%) and diabetes (28.21%). Since patients with chronic conditions are more susceptible to COVID-19 infection, they are at increased risk for complications such as deterioration of underlying diseases, pneumonia, failure of other organs, and sepsis; consequently, their chronic diseases may become severe, or the patients may die.[17] Our findings show that comorbidities may be risk factors for mortality in COVID-19-exposed patients.

Oxygen therapy administration has more of a possibility of recovery. Non-rebreathing oxygen mask (NRM) is the most common type of oxygen therapy used by patients. Despite this, NRM users had the highest mortality rate (52.5%) among all other groups. Invasive mechanical ventilation (IMV) has a positive effect on the recovery of many patients. Based on COVID-19 management guidelines in Indonesia, NRM is used as an initial therapy, but patients with acute respiratory failure were advised to be given IMV immediately.[18] In 2021–2022, Indonesia was confronted with two peaks of the Delta and Omicron variants of COVID-19. This period characterized increased hospitalizations and a lack of medical resources, such as oxygen supplies, IMV, and high-flow nasal cannula (HFNC). This led to a greater reliance on NRM in this study.[10] This study, in line with Ospina-Tascón et al.,[19] shows that patients receiving high-flow oxygen therapy showed earlier clinical recovery than conventional low-flow oxygen therapy.

On the other hand, administering antiviral, corticosteroid, and antibiotics to the patients can also recover, but the correlation was not significant. The remdesivir group accounted for the majority of patients who exhibited improvement. This is following management guidelines which recommend using remdesivir as a first-line antiviral for COVID-19.[18] The study conducted by Al-Ardhi et al.[20] revealed that remdesivir has significant advantages over other antivirals when administered to patients with severe to critical degrees. Remdesivir reduces the viral load in the body by inhibiting the RNA-dependent RNA polymerase (RdRp) enzyme, which is involved in the replication process of SARS-CoV-2.[21]

Dexamethasone has a positive effect, as indicated by the many patients who experience improvement. This follows the COVID-19 management guidelines, which recommend dexamethasone as the first-line corticosteroid, methylprednisolone, and hydrocortisone for patients receiving oxygen therapy who are severely ill.[18] According to a study, dexamethasone was the first drug to significantly reduce the risk of death in COVID-19 patients with respiratory failure requiring oxygen therapy.[22]

Besides cephalosporin and quinolone, some antibiotics like aminoglycoside and β-lactam are commonly used, especially in combination with

four antibiotics. These findings are consistent with the Indonesian Ministry of Health guidelines for selecting antibiotics for community pneumonia.[23] According to Risa et al.[24] found that up to 60% of COVID-19 patients with acute respiratory failure had a secondary bacterial infection; therefore, using empirical antibiotics is strongly advised, especially for ventilator-dependent patients.

The other drugs, like anticoagulants, anti-IL-6, vitamins, and additional treatments, didn't show a possibility of recovery. Anticoagulant enoxaparin was administered to 74 patients (61.7%). The majority of patients treated with enoxaparin died. Unfractionated heparin (UFH) and enoxaparin have demonstrated a lower mortality rate and a more significant number of improvements compared to other treatments. This is consistent with the COVID-19 management guidelines, which recommend enoxaparin for critically ill patients and UFH for those with moderate to severe symptoms.[18] Pawlowski et al.[25] discovered that the mortality rate and risk of bleeding in COVID-19 patients who received enoxaparin were significantly lower than in those who received UFH. This may account for the high use of enoxaparin in this study.

Only one patient was administered anti-IL-6 as tocilizumab, and no improvement was observed. Guidelines for managing COVID-19 in Indonesia and The Italian Society of Infectious and Tropical Disease (SIMIT) recommend using tocilizumab in patients with severe COVID-19 or acute respiratory failure.[18]

Most patients were given vitamins in the form of a combination of vitamin C, vitamin D, and multivitamins, and eight patients (6.7%) experienced improvement. It is in line with the management guidelines that recommend a combination of vitamin C, vitamin B1, and vitamin D.[18] Most of the patients in this study obtained vitamin B1 through the ingredients in the multivitamin. In addition to the standard treatment, four patients received intravenous immunoglobulin (2.5%) and convalescent plasma (0.8%). All patients who received adjunctive therapy perished.

The research has a limitation: this study did not assess treatment duration, which may have influenced treatment outcomes. Additional research analyzing the suitability of treatment relative to hospital standard operating procedures and the duration of treatment can confirm this finding.

## Conclusions

COVID-19 patients with acute respiratory failure were predominantly male, aged 30–60 years old, and had a hospital stay of 1 to 3 days. The majority presented with severe disease and at least one comorbidity. Pharmacological treatments commonly administered included antivirals, corticosteroids, anticoagulants, vitamins, and antibiotics. In addition to standard therapies, other interventions such as anti-IL-6, intravenous immunoglobulin (IVIG), and convalescent plasma were also employed. Potential therapies that may enhance patient recovery include oxygen therapy, antiviral agents, corticosteroids, and antibiotics.

## Conflict of Interest

The authors have declared that no conflict of interest exists.

## References

1. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: immunopathogenesis and immunotherapeutics. Signal Transduct Target Ther. 2020;5(1):128.
2. Burki T. The origin of SARS-CoV-2. Lancet Infect Dis. 2020;20(9):1018–9.
3. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020 [Internet]. Geneva: World Health Organization; 2020 [cited 2024 June 12]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
4. Surendra H, Elyazar IR, Djaafara BA, Ekawati LL, Saraswati K, Adrian V, et al. Clinical characteristics and mortality associated with COVID-19 in Jakarta, Indonesia: a hospital-based retrospective cohort study. Lancet Reg Health West Pac. 2021;9:100108.
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet.

2020;395(10229):1054–62.
6. Valent A, Dudoignon E, Ressaire Q, Dépret F, Plaud B. Three-month quality of life in survivors of ARDS due to COVID-19: a preliminary report from a French academic centre. Anaesth Crit Care Pain Med. 2020; 39(6):740–1.
7. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, evaluation, and treatment of coronavirus (COVID-19). [Updated 2023 Aug 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [2024 June 16]. Available from: ncbi.nlm.nih.gov/books/NBK554776.
8. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'cytokine storm' in COVID-19. J Infect. 2020;80(6):607–13.
9. Xu W, Sun NN, Gao HN, Chen ZY, Yang Y, Ju B, et al. Risk factors analysis of COVID-19 patients with ARDS and prediction based on machine learning. Sci Rep. 2021;11(1):2933.
10. Bhatraju PK, Ghassemieh BJ, Nichols M, Kim R, Jerome KR, Nalla AK, et al. COVID-19 in critically ill patients in the Seattle region—case series. N Engl J Med. 2020;382(21):2012–22.
11. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature. 2020;588(7837):315–20.
12. COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators. Clinical characteristics and day-90 outcomes of 4244 critically ill adults with COVID-19: a prospective cohort study. Intensive Care Med. 2021;47(1):60–73.
13. Zali A, Khodadoost M, Gholamzadeh S, Janbazi S, Piri H, Taraghikhah N, et al. Mortality among hospitalized COVID-19 patients during surges of SARS-CoV-2 alpha (B.1.1.7) and delta (B.1.617.2) variants. Sci Rep. 2022;12(1):18918.
14. Faes C, Abrams S, Van Beckhoven D, Meyfroidt G, Vlieghe E, Hens N; Belgian Collaborative Group on COVID-19 Hospital Surveillance. Time between symptom onset, hospitalisation and recovery or death: statistical analysis of Belgian COVID-19 patients. Int J Environ Res Public Health. 2020;17(20):7560.
15. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Eur Respir J. 2020;55(5):200054.
16. Djaharuddin I, Munawwarah S, Nurulita A, Ilyas M, Tabri NA, Lihawa N. Comorbidities and mortality in COVID-19 patients. Gac Sanit. 2021;35(Suppl 2):S530–2.
17. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. 2020;94:91–5.
18. Burhan E, Susanto AD, Isbaniah F, Nasution SA, Ginanjar E, Pitoyo CW, et al. Pedoman tatalaksana COVID-19. 4th Edition. Jakarta: PDPI, PERKI, PAPDI, PERDATIN, IDAI; 2022.
19. Ospina-Tascón GA, Calderón-Tapia LE, García AF, Zarama V, Gómez-Álvarez F, Álvarez-Saa T, et al. Effect of high-flow oxygen therapy vs conventional oxygen therapy on invasive mechanical ventilation and clinical recovery in patients with severe COVID-19: a randomized clinical trial. JAMA. 2021;326(21):2161–71.
20. Al-Ardhi FM, Novotny L, Alhunayan A, Al-Tannak NF. Comparison of remdesivir and favipiravir - the anti-COVID-19 agents mimicking purine RNA constituents. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2022;166(1):12–20.
21. Gordon CJ, Tchesnokov EP, Woolner E, Perry JK, Feng JY, Porter DP, et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. J Biol Chem. 2020;295(20):6785–97.
22. RECOVERY Collaborative Group; Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, et al. Dexamethasone in hospitalized patients with COVID-19. N Engl J Med. 202;384(8):693–704.
23. Peraturan Menteri Kesehatan Republik Indonesia Nomor 2406/MENKES/ PER/XII/2011 tentang Pedoman Umum Penggunaan Antibiotik.
24. Risa E, Roach D, Budak JZ, Hebert C, Chan JD, Mani NS, et al. Characterization of secondary bacterial infections and antibiotic use in mechanically ventilated patients

with COVID-19 induced acute respiratory distress syndrome. J Intensive Care Med. 2021;36(10):1167–75.
25. Pawlowski C, Venkatakrishnan AJ, Kirkup C, Berner G, Puranik A, O'Horo JC, et al. Enoxaparin is associated with lower rates of mortality than unfractionated Heparin in hospitalized COVID-19 patients. EClinicalMedicine. 2021;33:100774.

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**RESEARCH ARTICLE**

# Analysis of *FOXE1* rs3758249, *IRF6* rs2235375, *MTRR* A66G in Non-syndromic Cleft Lip and Palate among Indonesian Deutero-Malay Population

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**Abstract**

Non-syndromic cleft lip and palate (NS-CLP) is one of the most common orofacial malformations, with an incidence of 1 in 700 live births worldwide. This study aimed to determine the risk factor for NS-CLP among the Indonesian Deutero-Malay population by analyzing the *FOXE1* rs3758249, *IRF6* rs2235375, and *MTRR* A66G polymorphisms. It is a case-control study, using 50 samples of NS-CLP patients and 50 samples of control (for *FOXE1* rs3758249 and *MTRR* A66G), 30 samples of NS-CLP patients, and 30 samples of control (for *IRF6* rs2235375). After DNA was extracted, polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs) were performed by using restriction enzymes of Mscl (*FOXE1* rs3758249) and Nde1 (*MTRR* A66G), and sequencing was performed for *IRF6* rs2235375. The study was done in the Molecular Genetic Laboratory, Faculty of Dentistry Universitas Padjadjaran Bandung, from September 2023 to January 2024. The chi-square test was used with the exact Fisher's alternatives. The results showed that in *FOXE1* 3758249, A allele (mutant) was found more in control (OR=0.744, p>0.05), in *MTRR* A66G, G allele (mutant) was found more in NS-CLP (OR=1.267, p>0.05) meanwhile in *IRF6* rs2235375, G allele (mutant) (OR=1.710, p>0.05) was found more in NS-CLP. This study concluded that *FOXE1* rs 3758249, *IRF6* rs2235375, and *MTRR* A66G genes were not the risk factor for NS-CLP in the Indonesian Deutero-Malay population.

**Keywords:** Deutero-Malay, *FOXE1* rs3758249, Indonesian, *IRF6* rs2235375, *MTRR* A66G, non-syndromic cleft lip and palate

## Introduction

Cleft lip and palate (CLP) is a congenital orofacial malformation caused by the failure of the upper lip and palate to fuse.[1] Clinically, three main types of CLP include cleft lip (CL), which is a cleft that occurs only on the lip; cleft palate (CP), which is an aperture that occurs on the palate; and cleft lip and palate (CLP), which is a cleft that occurs on both the lip and palate.[2] CLP can be divided into syndromic (S) and non-syndromic (NS) depending on whether any other syndromes accompany CLP.[2]

The NS-CLP is thought to be influenced by genetic and environmental factors (multifactorial).[3] Genetic factors include polymorphisms in specific genes that are also associated with mechanisms that predispose to CLP.[2] Environmental factors can be influenced by alcohol consumption and smoking during pregnancy. In contrast, genetic factors occur when the patient's parents directly inherit the condition.[4]

Approximately 70% of CLP cases are NS-CLP.[5] The highest prevalence of NS-CLP is in Asian populations, followed by people of Western European descent and Africans.[6] Based on a study in West Java, Indonesia, from 2011 to 2015, using data collected from patients who received treatment at hospitals in that period, there were 1,596 patients with oral aperture, and it was found that NS-CLP patients had the highest percentage,

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reaching 50.53% from all cases. Furthermore, NS-CP was found in 25.05% of patients, while NS-CL occurred in approximately 24.42% of patients.[7]

The forkhead box E1 (*FOXE1*) gene is thought to be one gene involved in the development of human craniofacial clefts; it is a transcription factor involved in the growth of the thyroid gland and the formation of the craniofacial epithelium. *FOXE1* involvement has been reported in conditions such as congenital hypothyroidism, Van der Woude syndrome, Pierre Robin syndrome, and CLP. Several studies found a significant association between NS-CLP and single nucleotide polymorphisms (SNPs) or polymorphism of *FOXE1* rs3758249. Based on a study in the Northeast region of China, it was concluded that the *FOXE1* gene has a close relationship with NS-CLP.[8] The interferon regulatory factor 6 (*IRF6*) gene is involved in the immune response. It has functions in cell signaling, craniofacial morphogenesis, epithelial cell proliferation, and differentiation. It encodes for a transcription factor that regulates the expression of interferons and other genes necessary for the immune system and wound healing.[9] Polymorphisms in the *IRF6* gene can cause epidermal hyperproliferation, leading to impaired fusion in various lip and palate formation processes. These polymorphisms fail terminal differentiation (proliferation of epidermis or epithelium differentiating into mesenchyme) and multiple epithelial adhesions, leading to CLP. Studies in different populations, such as Europe and China, have identified the *IRF6* gene as one of the genes predisposing to NS-CLP and as a candidate gene that consistently exerts a significant influence on the incidence of NS-CLP, including *IRF6* rs2235375.[10] Methionine synthase reductase (*MTRR*) gene polymorphisms are also associated with the incidence of NS-CLP and being involved in folic acid metabolism, especially *MTRR* rs1801394 A66G.[11] A study in the Chinese population showed that the *MTRR* A66G gene was thought to be associated with an increased risk of NS-CLP.[12] However, a study in Turkey's population showed that *MTRR* A66G was not a risk factor for the incidence of NS-CLP.[13]

The *FOXE1* rs3758249, *IRF6* rs2235375, and *MTRR* A66G gene polymorphisms as risk factors of NS-CLP in the Indonesian Deutero-Malay population, which is the largest population in Indonesia, have never been studied before. Therefore, we are interested in analyzing *FOXE1* rs3758249, *IRF6* rs2235375, and *MTRR* A66G as risk factors of NS-CLP in the Indonesian Deutero-Malay population, which is the largest population in Indonesia.

## Methods

This study was done at the Molecular Biology Laboratory, Faculty of Dentistry, Universitas Padjadjaran Bandung, Indonesia, from September 2023 to January 2024. It was approved by the Research Ethics Committee of Universitas Padjadjaran with the number 1341/UN6.KEP/EC/2023.

The samples consist of 50 NS-CLP patients and 50 controls for *FOXE1* rs3758249 and *MTRR* A66G, 30 NS-CLP patients, and 30 controls for *IRF6* rs 2235375. The DNA was isolated from the venous blood of all samples, and then polymerase chain reaction (PCR) was done using primers that include 5'GA TGGTGGTGCCAGGTGA-3 (forward) and 5'GCTTTGAGCGTTTCCACA-3' (reverse) for *FOXE1* rs3758249,[14] 5'AGTTGGCCCAAAACTGAAC3' (forward) and 5'GGCTAGCCAGGAAACAGAAA3' (reverse) for *IRF6* rs2235375, and 5'- GCA AAG GCC CAT CGC AGA AGA CAT-3' (forward) and 5'- GTG AAG ATC TGC AGA AAA TCC ATG TA-3' (reverse) for *MTRR* A66G.[11]

The PCR results were evaluated by using agarose gel electrophoresis. The optimal PCR product for *FOXE1* rs3758249 is a band of 211 basepairs (bp), for *IRF6* rs2235373 is a band of 243 bp, and for *MTRR* A66G is a band of 66 bp. The optimal PCR products were then evaluated by using restriction fragment length polymorphisms (RFLPs), which were digested by restriction enzymes of Msc1 (*FOXE1* rs3758249),[8] and NdeI (*MTRR* A66G).[15] In contrast, the Sanger sequencing method sequenced the optimal PCR product of *IRF6* rs2235375. Allele and genotype frequencies between patients and control subjects were analyzed using the chi-square test, and Fisher's exact test will be used as another alternative.

## Results

The optimal PCR products of the *FOXE1* rs3758249, *IRF6* rs2235375, *MTRR* A66G, and

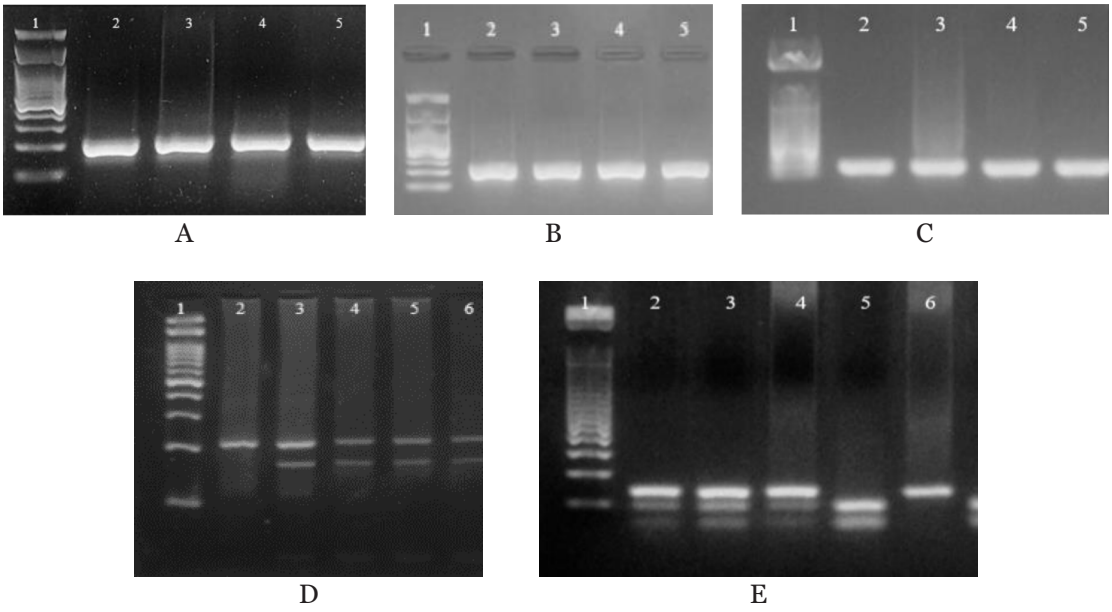


**Figure 1 Polymerase Chain Reaction (PCR) Products**

Note: (A) PCR product of *FOXE1* rs3758249, lane 2–5 DNA bands of optimal PCR products (211 bp); (B) PCR product of *IRF6* rs2235375, lane 2–5 DNA bands of optimal PCR products (243 bp); (C) PCR product of *MTRR* A66G, line 2–5 DNA bands of PCR products (66 bp); (D) PCR-RFLPs of *FOXE1* rs3758249, lane 2 and 7 GG genotype (homozygous normal), lane 3–6 GA genotype (mutant heterozygote); (E) PCR-RFLPs of *MTRR* A66G, lane 2–4 AG genotype (heterozygous mutant), lane 5 and 7 AA genotype (homozygous normal), lane 6 GG genotype (homozygous mutant)

PCR-RFLPs of *FOXE1* rs3758249 and *MTRR* A66G are shown in Figure 1. In Figure 1D, there were only two genotypes of *FOXE1* rs3758249, including the GG genotype (homozygous normal/wild type; 211 bp) and GA genotype (heterozygous mutant; 211, 163, and 48 bp). In Figure 1E, there were three genotypes of *MTRR* A66G that include AA genotype (homozygous normal/wild type; 44 and 22 bp), AG genotype (mutant heterozygous; 66, 44, and 22 bp), and GG genotype (mutant homozygous; 66 bp). To evaluate the PCR-RFLPs result of *FOXE1* rs3758249, some of the samples were assessed by sequencing the Sanger method from each genotype (Figure 2), but we did not do the sequencing of *MTRR* A66G due to very short base sequences. Sequencing results of *IRF6* rs2235373 are shown in Figure 3, and it shows the CC genotype (homozygous normal/wild type), CG genotype (mutant heterozygous), and GG genotype (mutant homozygous).

The allele and genotype frequencies are described in Tables 1, 2, and 3. The allele and genotype frequencies of all polymorphisms did not show risk factors of NS-CLP. It indicates that *FOXE1* rs3758249, *IRF6* rs2235375, and *MTRR* A66G are not risk factors for NS-CLP

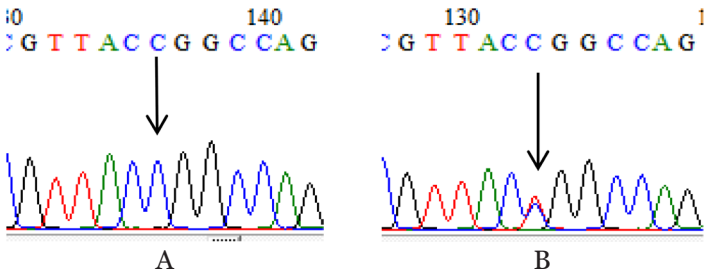


**Figure 2 Sequencing Results of *FOXE1* rs3758249**

Note: (A) GG genotype (normal homozygous/wild type); (B) GA genotype (mutant heterozygous)

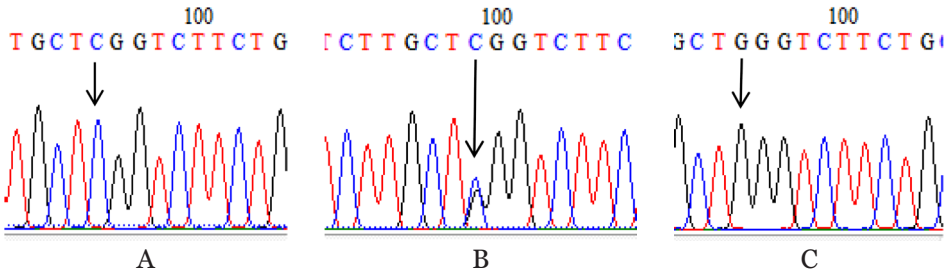


**Figure 3 Sanger Sequencing Results of *IRF6* rs2235375**
Note: (A) Normal homozygous CC genotype (normal homozygous/wild type); (B) CG genotype (mutant heterozygous); (C) GG genotype (mutant homozygous)

**Table 1 Allele and Genotype Frequency of the *FOXE1* rs3758249 among NS-CLP and Control**

| Allele and Genotype | NS-CLP n=50 | Control n=50 | $\chi^2$ | p | OR | 95% CI |
|---|---|---|---|---|---|---|
| G | 90 | 87 | 0.442 | 0.506 | 1.345 | 0.560–3.228 |
| A | 10 | 13 | 0.442 | 0.506 | 0.744 | 0.310–1.785 |
| GG | 40 | 37 | 0.508 | 0.476 | 1.405 | 0.550–3.590 |
| GA | 10 | 13 | 0.508 | 0.476 | 0.712 | 0.279–1.818 |
| AA | 0 | 0 | 0 | 0 | 0 | 0–0 |

Note: OR: odds ratio, CI: confidence interval, G: wild type allele of *FOXE1* rs3758249, A: mutant allele of *FOXE1* rs3758249, significance p<0.05

**Table 2 Allele and Genotype Frequency of the *IRF6* rs2235373 among NS-CLP and Control**

| Allele and Genotype | NS-CLP n=30 | Control n=30 | $\chi^2$ | p | OR | 95% CI |
|---|---|---|---|---|---|---|
| C | 26 | 34 | 2.133 | 0.144 | 0.585 | 0.284–1.204 |
| G | 34 | 26 | 2.133 | 0.144 | 1.710 | 0.831–3.521 |
| CC | 5 | 11 | 3.068 | 0.080 | 0.345 | 0.103–1.163 |
| CG | 16 | 12 | 1.071 | 0.301 | 1.714 | 0.616–4.772 |
| GG | 9 | 7 | 0.341 | 0.559 | 1.408 | 0.445–4.453 |

Note: OR: odds ratio, CI: confidence interval, C: wild type allele of *IRF6* rs2235373, G: mutant allele of *IRF6* rs2235373, significance p<0.05

**Table 3 Allele and Genotype Distribution of the *MTRR* A66G among NS-CLP and Control**

| Allele and Genotype | NS-CLP n=50 | Control n=50 | $\chi^2$ | p | OR | 95% CI |
|---|---|---|---|---|---|---|
| A | 67 | 72 | 0.590 | 0.443 | 0.790 | 0.432–1.444 |
| G | 33 | 28 | 0.590 | 0.443 | 1.267 | 0.693–2.316 |
| AA | 22 | 24 | 0.161 | 0.688 | 0.851 | 0.387–1.870 |
| AG | 23 | 24 | 0.040 | 0.841 | 0.923 | 0.421–2.024 |
| GG | 5 | 2 | 1.425 | 0.436 | 2.667 | 0.492–14.445 |

Note: OR: odds ratio, CI: confidence interval, A: wild type allele of *MTRR* A66G, G: mutant allele of *MTRR* A66G, significance p<0.05

in the Indonesian Deutero-Malay population. Meanwhile, GA genotype of *FOXE1* rs3758249 (OR=0.712, p=0.476) tend to be a protective factor and no AA genotype was found (Table 1). The G allele (OR=1.710, p=0.144) and CG genotype (OR=1.714, p=0.301) of *IRF6* rs2235373 tend to increase the risk of NS-CLP (Table 2). The GG genotype of *MTRR* A66G tends to be a risk factor for NS-CLP (OR=2.667, p=0.436, Table 3).

## Discussion

The *FOXE1* gene is located on chromosome 9q22.q33, consisting of one exon and expressed transiently in the thyroid gland and anterior pituitary, is part of a family of transcription factors containing a DNA-binding forkhead domain that can bind and open chromatin structures.[8,14] Polymorphisms of this gene, especially in the promoter region, have been associated with various thyroid-related etiologies, orofacial cleft including CLP, hypothyroidism (HT), and thyroid cancer, so the *FOXE1* rs3758249 gene has a very important role in the process of embryonic development. *FOXE1* rs3758249 polymorphism is in the form of base G into A substitution in the upstream region.[8,14] There are different results of the risk factors for *FOXE1* rs3758249 in the incidence of NS-CLP in several other populations. A study in Northeastern China showed that *FOXE1* rs3758249 was strongly associated with NS-CLP;[8] strong associations were found in Central Europe and Mayan Mesoamerican.[16] Meanwhile, another study on population in China showed that *FOXE1* rs3758249 was not associated with NS-CLP.[17] In this study, *FOXE1* rs3758249 is also not a risk factor for NS-CLP among the Indonesian Deutero-Malay population, but the GA genotype tends to be a protective factor (OR=0.712, p=0.476, Table 1). Differences in results on the same polymorphisms associated with NS-CLP revealed that the role of the same polymorphisms on NS-CLP depends on different races and geographical statuses.

The *IRF6* gene, also known as CLP gene,[18] is located on chromosome 1q32.2-q4 and encodes a member of IRF family and consists of 10 exons, with the start codon in exon three and the stop codon in exon.[8,10,19,20] *IRF6* plays an important role in epidermal development and is helpful for the expression of the leading ectoderm on the palatal shelves before and during primary palate formation. The *IRF6* mediates TGFβ3 in regulating epithelial-mesenchymal transformation (EMT) and apoptosis during palatal fusion. The *IRF6* gene regulates the degradation of Δp63 protein, resulting in the induction of p21 expression and apoptosis of medial edge epithelium (MEE), which are important for palatal fusion.[10] Mutations in *IRF6* result in hyperproliferation of the epidermis, which inhibits the terminal differentiation process so that the MEE in both palatal shelves fail to transform into mesenchyme in the EMT process and palatal shelves fusion does not occur then bring it into CP condition.[21] The study in Western China found an association of *IRF6* rs2235371, rs2013162, and rs2235375 polymorphisms with the incidence of NS-CLP abnormalities.[22] *IRF6* rs2235375 is located in the intron six as base C into G substitution. The study in Mexico detected a significant under-transmission of the common allele C and a significant over-transmission of the allele G for the rs2235375 marker, and the study in South India also detected *IRF6* rs2235375 to have a positive association with NS-CLP.[20,22,23]

Some *IRF6* polymorphisms have been studied among the Deutero-Malay population associated with NS-CP and NS-CLP, with significant results in Indonesia. Those polymorphisms are *IRF6* rs2235371 as a risk factor of NS-CP,[24] *IRF6* rs2013162,[25] and *IRF6* rs642961[26] as the risk factors of NS-CLP. In this study, *IRF6* rs2235375 is not a risk factor for NS-CLP in the Indonesian Deutero-Malay population, but the mutant G allele tended to increase the risk of NS-CLP (OR=1.710, p=0.144). *IRF6* is known to be a CLP gene,[18] but not all polymorphisms in this gene can be associated with the risk of NS-CLP, especially in the Indonesian Deutero-Malay population, suggesting the different roles of each SNPs in the *IRF6* gene.

The *MTRR* gene is located on chromosome 5p15.2-15.3 with a length of about 3.6 kb and encodes 698 amino acids with a molecular weight of about 77 kDa and has 15 exons.[11] The *MTRR* gene is linked to folic acid metabolism. It plays a vital role in homocysteine metabolism.[27] The *MTRR* gene is required for reductive methylation of vitamin B12 and is a gene that encodes the enzyme methionine synthase reductase, which plays a vital role in methionine metabolism and vitamin B12 regeneration.[11] *MTRR* A66G is the most common polymorphism in which adenine

is replaced by guanine, causing the substitution of isoleucine with methionine at codon 22.[11,28] This polymorphism involved in homocysteine metabolism associated explicitly with elevated plasma homocysteine concentration that may increase the predisposition of NS-CLP.[27] Homocysteine is formed from methionine metabolism, homocysteine must undergo methylation to be converted into methionine. Folic acid deficiency can disrupt the process of the folate-methionine reaction, where methionine is needed for protein formation. When there is a disturbance in methionine formation, the process of protein formation can not occur properly, which causes *MTRR* deficiency and brings into hyperhomocysteinemia, which is thought to affect fusion between the medial nasal process and maxillary process, resulting in NS-CLP.[29]

In this study, *MTRR* A66G was not a risk factor associated with NS-CLP. However, the GG genotype tends to increase the risk of NS-CLP among the Indonesian Deutero-Malay population (OR=2.667, p=0.436, Table 3). This result was supported by a study of the population in Turkey, which also showed no significant relationship between *MTRR* A66G gene polymorphism and the incidence of NS-CLP.[11] However, a different result was found in the Chinese population, as there was a significant association between the *MTRR* A66G and the incidence of NS-CLP.[12]

## Conclusion

*FOXE1* rs 3758249, *IRF6* rs 2235375, and *MTRR* A66G gene are not risk factors for NS-CLP in the Indonesian Deutero-Malay population.

## Conflict of Interest

The authors reported no potential conflict of interest.

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## References

1. Allori AC, Mulliken JB, Meara JG, Shusterman S, Marcus JR. Classification of cleft lip/palate: then and now. Cleft Palate Craniofac J. 2017;54(2):175–88.
2. Vyas T, Gupta P, Kumar S, Gupta R, Gupta T, Singh HP. Cleft of lip and palate: a review. J Family Med Prim Care. 2020;9(6):2621–5.
3. Yaqoob M, Mahmood F, Hanif G, Bugvi SM, Sheikh MA. Etiology and genetic factors in clefts of lip and/or palate reported at children's hospital, Lahore, Pakistan. Indian J Hum Genet. 2013;19(2):136–43.
4. Davies KJM, Richmond S, Medeiros-Mirra RJ, Abbas HH, Wilson-Nagrani CE, Davis MG, et al. The effect of maternal smoking and alcohol consumption on lip morphology. J Orthod. 2022;49(4):403–11.
5. Howe LJ, Lee MK, Sharp GC, Davey Smith G, St Pourcain B, Shaffer JR, et al. Investigating the shared genetics of non-syndromic cleft lip/palate and facial morphology. PLoS Genet. 2018;14(8):e1007501.
6. Ittiwut R, Siriwan P, Suphapeetiporn K, Shotelersuk V. Epidemiology of cleft lip with or without cleft palate in Thais. Asian Biomed. 2016;10(4):335–8.
7. Sjamsudin E, Maifara D. Epidemiology and characteristics of cleft lip and palate and the influence of consanguinity and socioeconomic in West Java, Indonesia: a five-year retrospective study. Int J Oral Maxillofac Surg. 2017;46(Suppl 1):69.
8. Liu K, Lu Y, Ai L, Jiao B, Yu J, Zhang B, et al. Association between FOXE1 and non-syndromic orofacial clefts in a northeastern Chinese population. Br J Oral Maxillofac Surg. 2015;53(8):705–10.
9. Kurniati M, Sosiawan A, Pramono RMC, Notopuro H, Nuraini I, A'yun Q. BMP4 SNP rs17563 T>C gene polymorphism on non-syndromic cleft lip/palate in an Indonesian population. J Int Dent Med Res. 2021;14(2):595–9.
10. Wu W, Hua L, Wang H, Hao J, Chen Y, Li F, et al. Presence of sequence and SNP variation in the IRF6 gene in healthy residents of Guangdong province. Open Life Sci. 2016;11(1):476–86.
11. Aşlar D, Taştan H. Prevalence of MTHFR, MTR and MTRR gene polymorphisms in Turkish patients with nonsyndromic cleft lip and palate. Gene Ther Mol Biol. 2014;16:115–29.

12. Wang W, Jiao XH, Wang XP, Sun XY, Dong C. MTR, MTRR, and MTHFR gene polymorphisms and susceptibility to nonsyndromic cleft lip with or without cleft palate. Genet Test Mol Biomarkers. 2016;20(6):297–303.
13. Smarius B, Loozen C, Manten W, Bekker M, Pistorius L, Breugem C. Accurate diagnosis of prenatal cleft lip/palate by understanding the embryology. World J Methodol. 2017;7(3): 93–100.
14. Mendieta-Zerón H, Jiménez-Rosales A, Pérez-Amado CJ, Jiménez-Morales S. *FOXE1* mutation screening in a case with cleft lip, hypothyroidism, and thyroid carcinoma: a new syndrome? Case Rep Genet. 2017;2017: 6390545.
15. Yadav U, Kumar P, Rai V. Distribution of methionine synthase reductase (MTRR) gene A66G polymorphism in Indian population. Indian J Clin Biochem. 2021;36(1):23–32.
16. Ludwig KU, Böhmer AC, Rubini M, Mossey PA, Herms S, Nowak S, et al. Strong association of variants around FOXE1 and orofacial clefting. J Dent Res. 2014;93(4):376–81.
17. Xiao WL, Jia KN, Yu G, Zhao N. Association between forkhead box E1 polymorphisms and risk of non-syndromic cleft lip with or without cleft palate: a meta-analysis. Orthod Craniofac Res. 2020;23(2):151–9.
18. Thompson J, Mendoza F, Tan E, Bertol JW, Gaggar AS, Jun G, et al. A cleft lip and palate gene, *Irf6*, is involved in osteoblast differentiation of craniofacial bone. Dev Dyn. 2019;248(3):221–32.
19. Soleymani M, Ebadifar A, Khosravi M, Esmaeilzadeh E, Khorram Khorshid HR. Association of rs2013162 and rs2235375 polymorphisms in *IRF6* gene with susceptibility to non-syndromic cleft lip and palate. Avicenna J Med Biotechnol. 2022;14(2):181–5.
20. Gurramkonda VB, Syed AH, Murthy J, Lakkakula BVKS. *IRF6* rs2235375 single nucleotide polymorphism is associated with isolated non-syndromic cleft palate but not with cleft lip with or without palate in South Indian population. Braz J Otorhinolaryngol. 2018;84(4):473–7.
21. Jugessur A, Rahimov F, Lie RT, Wilcox AJ, Gjessing HK, Nilsen RM, et al. Genetic variants in *IRF6* and the risk of facial clefts: single-marker and haplotype-based analyses in a population-based case-control study of facial clefts in Norway. Genet Epidemiol. 2008;32(5):413–24.
22. Huang Y, Wu J, Ma J, Beaty TH, Sull JW, Zhu L, et al. Association between *IRF6* SNPs and oral clefts in West China. J Dent Res. 2009;88(8):715–8.
23. Ibarra-Arce A, García-Álvarez M, Cortés-González D, Ortiz de Zarate-Alarcón G, Flores-Peña L, Sánchez-Camacho S, et al. *IRF6* polymorphisms in Mexican patients with non-syndromic cleft lip. Meta Gene. 2015;4:8–16.
24. Nasroen SL, Maskoen AM, Soedjana H, Hilmanto D, Gani BA. *IRF6* rs2235371 as a risk factor for non-syndromic cleft palate only among the Deutero-Malay race in Indonesia and its effect on the *IRF6* mRNA expression level. Dent Med Probl. 2022;59(1):59–65.
25. Nasroen SL, Tammama T, Darwis RS, Adil A, Rahmutia S, Maskoen AM, et al. The *IRF6* rs2013162 and *MTHFR* A1298C rs1801131 gene polymorphisms related to non-syndromic cleft lip and palate among Deutero-Malay in Indonesia. Cleft Palate Craniofac J. 2024;61(12):2009–16.
26. Nasroen SL, Maskoen AM, Soedjana H, Hilmanto D, Gani BA. The *IRF6* AP-2α binding site polymorphism relate to the severity of non-syndromic orofacial cleft of Indonesian patients. Minerva Dent Oral Sci. 2023;72(1):8–15.
27. Liao PY, Lee KH. From SNPs to functional polymorphism: the insight into biotechnology applications. Biochem Eng J. 2010;49(2):149–58.
28. Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene. 2014;533(1):11–20.
29. Škovierová H, Vidomanová E, Mahmood S, Sopková J, Drgová A, Červeňová T, et al. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci. 2016;17(10):1733.

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**RESEARCH ARTICLE**

# Associated Tinnitus Risk Factors in Patients with Type 2 Diabetes Mellitus

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**Abstract**

Tinnitus risk factors for type 2 diabetes mellitus (T2DM) are still under debate. Our study objective was to find tinnitus prevalence and principal risk factors in community-dwelling T2DM subjects. It was an analytical observational cross-sectional design study conducted from August to October 2023 at a public health center in West Jakarta. The consecutive non-random sampling method included 140 ambulatory T2DM study subjects. Tinnitus Screener and Tinnitus Handicap Inventory assessed tinnitus presence and severity. Age, sex, T2DM duration, fasting and 2-hour post-prandial blood glucose concentrations, lipid profile, blood pressure, and severity of hypertension were extracted from medical records. Data analysis was by chi-squared or Fisher exact tests, followed by multiple logistic regression analysis, with statistical significance set at $p<0.05$. The subjects' mean age was 54.71±5.33 years, and T2DM duration was 8.75±2.55 years. Tinnitus prevalence was 92 (65.17%), with 44 subjects (47.8%) having moderate tinnitus. Multivariable logistic regression findings: age (p=0.576), T2DM duration (p=0.116), total cholesterol (p=0.053), HDL-cholesterol (p=0.425), hypertension (p=0.046). Hypertension increased the risk of tinnitus by 2.289 times in T2DM subjects after adjusting for age, T2DM duration, and total and HDL cholesterol. Hypertension is the main tinnitus risk factor. The high tinnitus prevalence in our T2DM subjects requires regular screening for auditory function and control of blood pressure to minimize tinnitus risk in T2DM subjects.

**Keywords:** Community, diabetes mellitus, hypertension, risk factor, tinnitus

## Introduction

Diabetes mellitus (DM) is a serious chronic condition with characteristic hyperglycemia resulting from low blood insulin concentrations due to reduced insulin synthesis or ineffective insulin utilization.[1] Globally, over 90% of DM cases are of type 2 (T2DM).[1] According to the International Diabetes Federation (IDF) 2021 report, Indonesia occupied fifth rank, with the current 19.5 million diabetes mellitus cases being projected to increase to 28.6 million in 2045.[1] In T2DM, the hyperglycemia may result in microangiopathy causing the basement membrane of the capillaries to thicken. Microvascular changes might also occur in the inner ear, disrupting the circulation flow and resulting in the narrowing of capillaries and the loss of the outer hair cells, which are responsible for the amplification of sound entering the cochlea.[2] This may lead to degeneration of nerve cells, observable in both the cochlea and the eighth cranial nerve, and atrophy of the outer hair cells in the organs of Corti, such that patients with T2DM frequently have hearing disorders, including tinnitus.[2]

Tinnitus is the uni- or bilateral occurrence of abnormal aural sounds without external sounds.[3] Little has been understood of the relationship between diabetes and tinnitus.[2] The metabolism of the inner ear depends on the oxygen and glucose supply from the blood circulation. Alterations in the glucose metabolism as a result of microangiopathy can disturb the function of the brain cells and the inner ear, which may lead to tinnitus.[2] Tinnitus prevalence in T2DM cases varies between countries from 9.2% to 26.4%.[3,4]

Several studies that have been conducted to

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determine the relationship between risk factors and the occurrence of tinnitus have shown inconsistent results. Panahi et al.,[5] Biswas et al.,[6] and Jarach et al.[7] confirmed that aging increases tinnitus prevalence. However, Oosterloo et al.[8] found that tinnitus may not be age-dependent. Mousavi et al.[3] and Dhulipalla et al.[9] showed a relationship between T2DM duration and tinnitus severity. Musleh et al.[10] reported that T2DM cases with high lipid profile showed a 2.2-fold significantly greater risk for tinnitus, whereas Lee et al.[11] determined that in the hypertriglyceridemia group, there was a 1.27-fold significant risk for tinnitus than in the non-hypertriglyceridemia group, after adjusting for other factors. Ramatsoma and Patrick[12] reported hearing loss and tinnitus were more frequently found in hypertensive than non-hypertensive adults. However, Huang et al.[13] showed that the odds of prior hypertension were similar between the groups with and without tinnitus.

Up to the present, information on prevalence and tinnitus risk factors in community-dwelling T2DM cases still needs to be improved. It is urgently required to plan essential T2DM policies to prevent tinnitus. In T2DM with chronic tinnitus, psychological or emotional effects, sleep disturbance, auditory dysfunction, and other health issues reduce the quality of life.[14] The present study aimed to determine the prevalence of tinnitus and the associated risk factors that may predict tinnitus occurrence in community-dwelling T2DM cases.

## Methods

This analytical observational cross-sectional study was conducted on ambulatory T2DM cases at a public health center in Grogol Petamburan, West Jakarta, from August to October 2023. A total of 140 patients with diabetes mellitus were collected by consecutive non-random sampling. The inclusion criteria for prospective subjects were: T2DM cases between 45–60 years old, T2DM duration >5 years, capable of good communication, and agreeing to become study subjects by giving written informed consent. The exclusion criteria were: consumption of ototoxic medications in the recent past, having neurological disorders, or having been diagnosed with inner or outer ear disease, acoustic trauma, head trauma, or chronic otitis media.

The sample size was computed using (1) the formula for an infinite (unknown) population and (2) the formula for a finite (known) population:

$$n_o = \frac{(Z\alpha^2) \times p \times q}{d^2} \quad .....(1)$$

Where $n_o$: required optimal sample size; Zα: 1.96; p: prevalence of tinnitus in diabetes mellitus = 0.09 (8); q: (1–p) = 0.91; determined degree of confidence or accuracy of measurement = 0.01; resulting in $n_o = 126$.

$$n = n_o/(1+(n_o/N)) \quad .....(2)$$

Because the number of persons with T2DM (N) at the data collection site was 568, using formula (2) and adding 15 percent of anticipated dropouts, the required sample size was 121. The final sample size was 140.

This study was approved for ethical clearance by the Research Ethics Committee of the Faculty of Medicine, Universitas Trisakti (148/KER-FK/VII/2023). Data on age, sex, T2DM duration, history of hypertension, current blood pressure, lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol), and fasting and 2-hour post-prandial blood glucose were obtained from medical records. To differentiate between tinnitus and the normal auditory phenomenon of "transient ear noise," the Tinnitus Screener was used.[15] Tinnitus Screener results of constant or intermittent tinnitus indicate chronic tinnitus. Subsequently, the tinnitus status was determined with the Tinnitus Handicap Inventory questionnaire (Indonesian version),[16] consisting of 26 items on tinnitus status. Initially, the subject was asked to answer each item with a three-level score (yes: four scores, occasionally: two scores, and no: 0 scores). Next, all scores were summed to obtain the total score. The range of obtainable scores was 0–100. The severity of tinnitus based on the total score was categorized into slight, mild, moderate, severe, and catastrophic, corresponding to score ranges of 0–16, 18–36, 38–56, 58–76, and 78–100, respectively.

Age was categorized into <50 years and ≥50 years, sex was categorized into male and female, and T2DM duration was categorized into <8 years and ≥8 years. Lipid profile (total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol) was categorized based on the

criteria of the Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, or ATP III).[17] Total cholesterol was categorized into <200 mg/dl and ≥200 mg/dl, triglyceride concentration into <150 mg/dl and ≥150 mg/dl, HDL cholesterol into <60 mg/dl and ≥60 mg/dl, and LDL cholesterol into <160 mg/dl and ≥160 mg/dl. Blood glucose categories were determined based on the criteria of the American Diabetes Association,[18] where fasting blood glucose was categorized into <120 mg/dl and ≥120 mg/dl. In contrast, 2-hour post-prandial blood glucose was categorized into <200 mg/dl and ≥200 mg/dl. The presence of hypertension was categorized into no hypertension if the blood pressure was <140/90 mmHg and hypertension if the blood pressure was ≥140/90 mmHg.

Before data analysis, data cleaning was performed using consistency, range, and logical checks. The Kolmogorov-Smirnov test for all numerical variables determined the normality of data distribution. Numerical data of normal distribution were presented as mean±SD, whereas numerical data of non-normal distribution were presented as median. Categorical data were reported as number of respondents (n), percentage (%), odds ratio (OR), and 95% confidence interval (95%CI). The t-test and Mann-Whitney test were used to find differences in numerical data between the tinnitus and non-tinnitus groups. Chi-squared test and Fisher's exact test were used to determine the relationship of the categorical data of age, sex, T2DM duration, lipid profile (total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol), blood glucose (fasting and 2-hour post-prandial), and presence of hypertension accompanied by tinnitus in T2DM cases. Risk factors with a p-value of <0.2 in the Mann-Whitney U-test and independent t-test were deemed candidate variables for analysis with the multivariable logistic regression test, with the statistical significance set at p<0.05. Data processing was done using the SPSS statistical program version 25.

## Results

This study involved 140 T2DN cases, with a mean age of 54.71±5.33 years. The majority of subjects (78 or 55.7%) were female, with a mean T2DM duration of 8.75±2.55 years.

**Table 1 Subject Characteristics**

| Variables | Tinnitus | | p[$] |
|---|---|---|---|
| | Present n=92 | Absent n=48 | |
| Age (years), median (min–max) | 56 (38–70) | 52.5 (41–68) | 0.005[*] |
| Duration of type 2 diabetes mellitus (years) | 10 (5–15) | 7.5 (5–14) | 0.004[*] |
| Blood lipids (mg/dl) | | | |
| Total cholesterol | 197.5 (110–291) | 202.5 (113–303) | 0.740 |
| Triglycerides | 146.59±60.16 | 154.88±77.93 | 0.138[#] |
| High-density lipoprotein cholesterol | 43 (25–76) | 43 (28–80) | 0.616 |
| Low-density lipoprotein cholesterol | 110.5 (40–181) | 89.5 (39–180) | 0.121 |
| Blood glucose (mg/dl) | | | |
| Fasting | 145.5 (77–379) | 144.5 (90–458) | 0.711 |
| 2-hour post-prandial | 198 (100–437) | 187 (98–487) | 0.970 |
| Blood pressure, mean±SD (mmHg) | | | |
| Systolic | 129 (100–169) | 120 (100–160) | 0.053 |
| Diastolic | 90 (70–100) | 80 (70–100) | 0.510 |
| Severity of tinnitus, n (%) | | | |
| Slight | 7 (7.6) | | |
| Mild | 28 (30.4) | | |
| Moderate | 44 (47.8) | | |
| Severe | 13 (14.1) | | |
| Catastrophic | 0 (0) | | |

Note: [$]Mann-Whitney U test, [#]independent t-test, [*]significance p<0.05. Hypertension categories: no hypertension = blood pressure <140 mmHg/80 mmHg, hypertension = blood pressure ≥140/80 mmHg. Severity of tinnitus was measured with the Tinnitus Handicap Inventory questionnaire and categorized into slight (score ≤16), mild (18–36), moderate (38–56), severe (58–76), catastrophic (78–100)

The subjects with and without hypertension were equal, namely 70 subjects (50%) in each group. Tinnitus was found in 92 subjects (65.17%), among whom 44 subjects (47.8%) had moderate tinnitus (Table 1).

The group of respondents with tinnitus had a significantly higher median age (p<0.005) and a substantially longer duration of T2DM (p<0.004) in comparison with the group of respondents without tinnitus. No differences were found in blood lipid (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol) and blood glucose levels (fasting and 2-hour post-prandial), as well as in blood pressure (systolic and diastolic) in the tinnitus and non-tinnitus groups (Table 1).

We used the chi-squared and Fisher's exact tests to determine the risk factors meeting the conditions for inclusion in the multivariate test (Table 2). We found five variables meeting the conditions for inclusion in the multivariate test, namely age (OR=0.364, 95% CI=0.153–0.869, p=0.020), T2DM duration (OR=0.373, 95% CI=0.180–0.773, p=0.007), total cholesterol (OR=0.619, 95% CI=0.306–1.250, p=0.180), HDL cholesterol (OR=0.528, 95% CI=0.199–1.405, p=0.196), and presence of hypertension (OR=2.167, 95% CI=1.060–4.430, p=0.033).

Table 3 shows the results of the multivariable

**Table 2 Relationship of Several Risk Factors with Tinnitus in T2DM Subjects**

| Variables | Tinnitus | | p[b] | OR (95% CI) |
|---|---|---|---|---|
| | Yes n=92 (%) | No n=48 (%) | | |
| Age (years) | | | | |
| <50 | 12 (46.2) | 14 (53.8) | 0.020[@] | 0.364 (0.153–0.869) |
| ≥50 | 80 (70.2) | 34 (29.8) | | |
| Gender | | | | |
| Male | 42 (67.7) | 20 (32.3) | 0.652 | 1.176 (0.581–2.381) |
| Female | 50 (64.1) | 28 (35.9) | | |
| Type 2 diabetes mellitus duration (years) | | | 0.007[@] | 0.373 (0.180–0.773) |
| <8 | 25 (51.0) | 24 (49.0) | | |
| ≥8 | 67 (73.6) | 24 (26.4) | | |
| Total cholesterol level (mg/dl) | | | | |
| <200 | 55 (70.5) | 23 (29.5) | 0.180[@] | 0.619 (0.306–1.250) |
| ≥200 | 37 (59.7) | 25 (40.3) | | |
| Triglycerides level (mg/dl) | | | | |
| <150 | 49 (85.3) | 26 (34.7) | 0.919 | 1.037 (0.515–2.089) |
| ≥150 | 43 (66.2) | 22 (33.8) | | |
| High-density lipoprotein cholesterol level (mg/dl) | | | 0.196 | 0.528 (0.199–1.405) |
| <60 | 82 (67.8) | 39 (32.2) | | |
| ≥60 | 10 (52.6) | 9 (47.4) | | |
| Low-density lipoprotein cholesterol level (mg/dl) | | | 1.000 | 1.048 (0.299–3.673) |
| <160 | 84 (65.6) | 44 (34.4) | | |
| ≥160 | 8 (66.7) | 4 (33.3) | | |
| Fasting blood glucose level (mg/dl) | | | | |
| <126 | 26 (63.4) | 15 (36.9) | 0.712 | 1.154 (0.539–2.469) |
| ≥126 | 66 (66.7) | 33 (33.3) | | |
| 2-hour post-prandial blood glucose level (mg/dl) | | | 0.736 | 1.128 (0.559–2.277) |
| <200 | 49 (64.5) | 27 (35.5) | | |
| ≥200 | 43 (67.2) | 21 (32.8) | | |
| Hypertension | | | | |
| No hypertension | 40 (57.1) | 30 (42.9) | 0.033[@] | 2.167 (1.060–4.430) |
| Hypertension | 52 (74.3) | 18 (25.7) | | |

Note: [b]chi-squared test and Fischer's exact test; [@]p<0.2: meet conditions for inclusion in multivariable multiple logistic regression analysis, significance p<0.05

**Table 3 Results of Multivariable Logistic Regression Analysis**

| Variables | aOR | 95% CI | p |
|---|---|---|---|
| Age (years) | 1.358 | 0.465–3.971 | 0.576 |
| Type 2 diabetes mellitus duration (years) | 1.990 | 0.844–4.696 | 0.116 |
| Total cholesterol level (mg/dl) | 0.543 | 0.248–1.189 | 0.127 |
| High-density lipoprotein cholesterol level (mg/dl) | 0.652 | 0.229–1.862 | 0.425 |
| Hypertension | 2.289 | 1.015–5.163 | 0.046* |

Note: aOR: adjusted odds ratio, *significance p<0.05

multiple logistic regression analysis after adjusting for age, T2DM duration, total cholesterol, and HDL cholesterol. Hypertensive T2DM subjects had a significantly greater tinnitus risk of 2.289 times that of non-hypertensive T2DM subjects (aOR=2.289, 95% CI=1.015–5.163, p=0.046).

## Discussion

Studies on tinnitus prevalence in T2DM have previously been conducted in many countries, but only a few studies have determined tinnitus prevalence and risk factors in community-dwelling T2DM cases. More than half of our respondents (65.17%) had tinnitus (Table 1). In this study, both the prevalence and severity of tinnitus in T2DM subjects were more significant than in studies conducted in Iran and Malaysia, which found tinnitus prevalences of 26.4% and 9.1%, respectively.[3,4] The differences in prevalences between our study and those of the Iranian and Malaysian studies may have been caused by the different demographic and clinical characteristics of the study subjects.

Our study results show that younger subjects aged <50 years had a 0.364 times significantly lower risk for tinnitus than older subjects aged ≥50 years (Table 2). The mechanisms that may explain the relationship between older age and the occurrence of tinnitus are still unclear to the present. Several studies confirm that tinnitus prevalence significantly increases with increasing age.[5–7] Each increment in age by 10 years from the age of 45 years onwards raises the ORs to 1.58, 2.84, and 3.24, respectively.[5] In a general elderly population, 1 in 5 persons has tinnitus, in which participants with hearing impairment are twice as likely to have tinnitus.[8] Tinnitus is not age-dependent, even in conjunction with the age-dependent presence of hearing impairment.[8]

The National Health and Nutrition Examination Survey (NHANES) data show that an increment in hearing loss by one decibel increases the odds of tinnitus by 3% in younger persons but by 6% in older persons. NHANES indicated that aging could make individuals more vulnerable to developing tinnitus when hearing loss is present.[19] Therefore, for a specific rise in hearing loss, older persons will report more tinnitus than younger persons.[19] Because the underlying mechanisms remain unclear, it is suggested that hearing damage may accelerate brain aging, which may be the actual tinnitus factor.[20]

Unlike hearing impairment, tinnitus is probably not associated with aging processes. One possible explanation is that the pathophysiology of age-related hearing impairment is principally different from other types of hearing loss that are more likely to induce tinnitus.[21] It is certain that tinnitus and hearing impairment in older people co-occur. Still, the age-related aspect of hearing impairment does not contribute to the association between hearing impairment and tinnitus.[8]

Our study results show that a T2DM duration of <8 years poses a 0.373 times significantly lower risk for tinnitus than a T2DM duration of ≥8 years (Table 2). A longer duration of suffering from T2DM is associated with an increased hazard ratio at risk of moderate or high hearing loss in comparison with non-T2DM cases (pooled multivariable-adjusted hazard ratio [HR] 1.24 [95% CI=1.10, 1.40]).[22] Other studies by Mousavi et al.[3] and Dhulipalla et al.[9] showed an association between T2DM duration of more than ten years and tinnitus.

Among the hypotheses explaining the relationship between the duration of T2DM and tinnitus, one hypothesis is that T2DM generally occurs in persons of advanced age. The longer a person has T2DM, the older that person becomes. In addition, the presence of chronic

hyperglycemia leads to a higher probability of microangiopathy as a complication of T2DM.[2] The metabolism of the inner ear depends on the oxygen and glucose supply from the blood circulation. Alterations in glucose metabolism as a result of microangiopathy in the inner ear can disturb the function of the brain cells and the inner ear, which may contribute to tinnitus.[2] Degraded hearing may exacerbate this due to neurosensory impairments that increase with age.[3] In one study, it was shown that tinnitus is not age-dependent, but that aging may render a person more vulnerable to developing tinnitus when hearing loss is present.[8,19] The dependency between age and tinnitus may be due to T2DM duration because the longer the T2DM duration, the more severe the T2DM side effects.[3]

Our bivariate analysis determined that there was no significant relationship between lipid profiles in the groups with tinnitus and without tinnitus (Table 2). The development of tinnitus, hearing loss, and vertigo may be significantly influenced by high blood total cholesterol, LDL cholesterol, and triglyceride concentrations, causing blockage of the inner ear microcirculation by microthrombosis, increased blood viscosity, or altered vasomotion, ultimately resulting in reduced cochlear perfusion.[10,23] Our results differ from those of Musleh et al.[10] and Lee et al.[11] Musleh et al.[10] showed that subjects with a high lipid profile had a statistically significant two-fold risk for tinnitus (OR=2.2, p=0.024). Lee et al.[11] found that the OR of tinnitus in subjects with hypertriglyceridemia was 1.27-fold that in subjects without hypertriglyceridemia after adjusting for age, sex, hypertension, diabetes, dyslipidemia, anemia, current smoking, obesity, noise exposure, stress cognition, and depressive mood or anxiety (95% CI=1.04–1.56, p=0.022). The differences between our study and the studies of Musleh et al.[10] and Lee et al.[11] may have been caused by the respondents' dietary patterns and lifestyles, which were not investigated in the present study. However, our study did not find differences in lipid levels (total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol) between the tinnitus and non-tinnitus groups (Table 1).

The multivariate analysis of our study showed that subjects with T2DM and hypertension have a 2.289 times greater risk for tinnitus than do subjects with T2DM without hypertension (aOR=2.289, 95% CI=1.015–5.163, p=0.046). Tinnitus may more frequently occur concurrently with auditory or systemic disorders such as T2DM and hypertension.[3] The action of hypertension and T2DM on the auditory system may be explained by the assumption that high blood pressure and hyperglycemia increase blood viscosity, thereby increasing resistance to blood flow, thus depriving the tissues of oxygen.[2,4] The interference with inner ear functions may ultimately be caused by diabetes mellitus or hypertension manifesting as hyperviscosity or microangiopathy syndrome.[25]

The reported relationships of hypertension and diabetes with tinnitus have been controversial. In patients with hypertension, the probability of tinnitus increases (OR=1.46, 95% CI=1.25–1.70),[26] and the probability of hearing loss compared to patients without hypertension.[12] T2DM subjects with one and ≥2 comorbidities had significant two-fold and three-fold greater odds of tinnitus (OR=2.03, 95% CI=1.70–2.43 and OR=3.24, 95% CI=2.62–4.01, respectively) as compared with T2DM subjects without comorbidities.[5] These results are consistent with our study results; however, differing results were shown by NHANES and the Korea National Health and Nutrition Examination Survey (KNHANES),[26,27] showing that hypertension and diabetes mellitus did not increase the odds of frequent tinnitus. KNHANES analysis instead found that a history of hyperlipidemia was associated with tinnitus.[27] These contradictory study results indicate the need for prospective studies to investigate the abovementioned relationships.

In our study, the greater tinnitus prevalence in T2DM cases merits consideration because chronic tinnitus negatively impacts the quality of life of T2DM cases due to psychological or emotional effects, sleep disturbance, and auditory and health effects.[14] Tinnitus should be considered a public health problem and should be included in the planning design at all levels of care, including primary care in the community setting. Auditory function disorders must be diagnosed much earlier so that timely intervention strategies may be instituted. Screening for hearing in T2DM is essential for successful prevention. It should be performed at least every 2 years or sooner if high-risk conditions exist, such as tinnitus perception and poor speech understanding.[28] Our study results confirm that in T2DM, there is a need not only for glycemic control to prevent

complications[29] but also for screening of auditory functions because patients with T2DM are susceptible to tinnitus and hearing disorders. Impaired glucose metabolism and hypertension in patients with T2DM may cause microvascular complications in the inner ear, causing them to have more chances of hearing loss, tinnitus, and dizziness.[2,5,30]

Our study has several limitations such as (i) the cross-sectional study design that prevents the establishment of a cause-and-effect relationship between various factors and tinnitus; (ii) the Tinnitus Screener questionnaire that has not been validated for Indonesian patients and the fact that supporting examinations are still needed to confirm the diagnosis; (iii) the data on lipid and blood glucose levels that in the present study were obtained from medical records and are known to be affected by time-dependent variable dietary intakes.

Future large-scale prospective and longitudinal studies should clarify the link between lipid profiles, Hba1c, and tinnitus. The multi-causal contributing factors of tinnitus must still be studied in elderly people, presumably comprising otologic, metabolic, neurologic, psychological, and cardiovascular conditions, as well as medications.

## Conclusions

Hypertension is the most influential risk factor for tinnitus in patients with T2DM. The high prevalence of tinnitus in T2DM shows the need for routine hearing screening to detect tinnitus as early as possible. Simultaneous blood pressure and glycemic control are necessary to minimize the risk of tinnitus, particularly in patients with T2DM and comorbid hypertension.

## Conflict of Interest

The authors do not have any conflict of interest to declare.

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## References

1. International Diabetes Federation. IDF Diabetes Atlas. 10th Edition. Brussels: International Diabetes Federation; 2021.
2. Taneja N. Tinnitus, hearing impairment and diabetes: a mini-review. Otolaryngol Open J. 2017;Spec 5:S6–9.
3. Mousavi SHG, Sajadinejad B, Khorsandi S, Farhadi A. Diabetes mellitus and tinnitus: an epidemiology study. Maedica (Bucur). 2021;16(4):580–4.
4. Baharudin AS, Kiat NC, Isahak N, Xin TW, Zhaki FNA, Rasyid NA, et al. Prevalence of tinnitus in type II diabetes mellitus with or without hypertension patients in Universiti Kebangsaan Malaysia Medical Centre. Int Med J. 2017;24(5):398–401.
5. Panahi R, Jalali MM, Joukar F, Maroufizadeh S, Naghipour M, Mansour-Ghanaei F. Prevalence of tinnitus and its associated factors in the PERSIAN Guilan Cohort Study. Iran J Otorhinolaryngol. 2023;35(126):29–38.
6. Biswas R, Lugo A, Akeroyd MA, Schlee W, Gallus S, Hall DA. Tinnitus prevalence in Europe: a multi-country cross-sectional population study. Lancet Reg Health Eur. 2022;12:100250.
7. Jarach CM, Lugo A, Scala M, van den Brandt PA, Cederroth CR, Odone A, et al. Global prevalence and incidence of tinnitus: a systematic review and meta-analysis. JAMA Neurol. 2022;79(9):888–900.
8. Oosterloo BC, Croll PH, Baatenburg de Jong RJ, Ikram MK, Goedegebure A. Prevalence of tinnitus in an aging population and its relation to age and hearing loss. Otolaryngol Head Neck Surg. 2021;164(4):859–68.
9. Dhulipalla S, Makkena A, Gowthami B, Sravani B. A cross sectional study of prevalence of hearing impairment and tinnitus in type 2 diabetes mellitus patients. EJMCM. 2023;10(4):976–82.
10. Musleh A, Alshehri S, Qobty A. Hyperlipidemia and its relation with tinnitus: cross-sectional approach. Niger J Clin Pract. 2022;25(7):1046–9.
11. Lee HJ, Lee DC, Kim CO. The association between serum lipid levels and tinnitus prevalence and severity in korean elderly: a nationwide population-based cross-sectional study. Yonsei Med J. 2024;65(3):156–62.
12. Ramatsoma H, Patrick SM. Hypertension associated with hearing loss and tinnitus among hypertensive adults at a tertiary

hospital in South Africa. Front Neurol. 2022;13:857600.
13. Huang PH, Xirasagar S, Chen JH, Cheng YF, Kuo NW, Lin HC. Absence of association of tinnitus with pre-existing hypertension: a population-based study. Ann Otol Rhinol Laryngol. 2023;132(7):756–62.
14. Swain SK. Impact of tinnitus on quality of life: a review. Int J Adv Med. 2021;8(7):1006–10.
15. Henry JA, Griest S, Zaugg TL, Thielman E, Kaelin C, Galvez G, et al. Tinnitus and hearing survey: a screening tool to differentiate bothersome tinnitus from hearing difficulties. Am J Audiol. 2015;24(1):66–77.
16. Bashiruddin JE, Alviandi W, Reinaldo A, Safitri ED, Pitoyo Y, Ranakusuma RW. Validity and reliability of the Indonesian version of tinnitus handycap inventory. Med J Indones. 2015;24(1):36–42.
17. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143–421.
18. American Diabetes Association Professional Practice Committee. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2022. Diabetes Care. 2021;45(Suppl 1):S17–38.
19. Reisinger L, Schmidt F, Benz K, Vignali L, Roesch S, Kronbichler M, et al. Ageing as risk factor for tinnitus and its complex interplay with hearing loss—evidence from online and NHANES data. BMC Med. 2023;21(1):283.
20. Cole JH, Marioni RE, Harris SE, Deary IJ. Brain age and other bodily 'ages': implications for neuropsychiatry. Mol Psychiatry. 2019; 24(2):266–81.
21. Langguth B, Landgrebe M, Schlee W, Schecklmann M, Vielsmeier V, Steffens T, et al. Different patterns of hearing loss among tinnitus patients: a latent class analysis of a large sample. Front Neurol. 2017;8:46.
22. Gupta S, Eavey RD, Wang M, Curhan SG, Curhan GC. Type 2 diabetes and the risk of incident hearing loss. Diabetologia. 2019; 62(2):281–5.
23. Avcı D. Increased serum lipid levels in patients with subjective tinnitus. Iran J Otorhinolaryngol. 2021;33(114):31–6.
24. Bachor E, Selig YK, Jahnke K, Rettinger G, Karmody CS. Vascular variations of the inner ear. Acta Otolaryngol. 2001;121(1):35–41.
25. de Moraes Marchiori LL, de Almeida Rego Filho E, Matsuo T. Hypertension as a factor associated with hearing loss. Braz J Otorhinolaryngol. 2006;72(4):533–40.
26. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. Am J Med. 2010; 123(8):711–8.
27. Kim HJ, Lee HJ, An SY, Sim S, Park B, Kim SW, et al. Analysis of the prevalence and associated risk factors of tinnitus in adults. PLoS One. 2015;10(5):e0127578.
28. Spankovich C, Yerraguntla K. Evaluation and management of patients with diabetes and hearing loss. Sem Hear. 2019;40(4):308–14.
29. Yenny Y, Herwana E, Wratsangka R. Skor risiko diabetes mellitus berkorelasi dengan kadar gula darah puasa: skrining diabetes mellitus tipe-2 pada masyarakat. Jurnal AKAL. 2022;3(2):193–207.
30. Kumar P, Singh NK, Apeksha K, Ghosh V, Kumar RR, Kumar Muthaiah B. Auditory and vestibular functioning in individuals with type-2 diabetes mellitus: a systematic review. Int Arch Otorhinolaryngol. 2022; 26(2):e281–8.

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**RESEARCH ARTICLE**

# The Impact of Social Value Orientation on Pro-environmental Behavior

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**Abstract**

Promoting pro-environmental behavior is a significant concern nowadays. Researchers have identified social value orientation as one of the key factors influencing pro-environmental behavior. This study aims to investigate the influence of social value orientation on pro-environmental behavior and to highlight differences based on gender. The research method used is non-experimental quantitative causality. This research was carried out in 2024 at B University in Bandung. The study participants were 378 students from B University in Bandung, selected using convenience sampling. The measurement tools used were the Triple Dominance Scale by Van Lange (1998) to assess social value orientation (SVO) tendencies and the GEB Scale by Kaiser (2020) to measure general ecological behavior, i.e., the tendency to engage in pro-environmental or non-pro-environmental behavior. Data analysis was done using JASP version 19. The results showed a significant influence of social value orientation on pro-environmental behavior with Fisher's exact test $p=0.017$ ($p<0.05$). An odds ratio of 0.084 was obtained, indicating that participants categorized as pro-social are 11.9 times more likely to engage in pro-environmental behavior than those classified as pro-self. Regarding gender differences, the chi-square test revealed no significant differences between males and females in terms of social value orientation tendencies with $\chi^2=0.056$ ($p>0.05$), as well as in terms of engaging in pro-environmental behavior, with $\chi^2=0.774$ ($p>0.05$). The conclusions of this study are that social value orientation affects pro-environmental behavior, and there was no significant difference between gender in social value orientation tendencies and pro-environmental behavior.

**Keywords:** Gender differences, pro-environmental behavior, social value orientation, students

## Introduction

Promoting pro-environmental behavior is an essential concern nowadays, as environmental degradation poses a significant threat to the well-being of our planet and its inhabitants.[1] Individual involvement in embodying pro-environmental behavior is critical to addressing environmental challenges and ensuring our planet's and future generations' long-term health and well-being. As future leaders and decision-makers, students play a crucial role in driving sustainable change.[2] Researchers have identified social value orientation as a key factor influencing pro-environmental behavior, and understanding this relationship is essential for developing effective interventions to encourage environmentally responsible action.[3]

Pro-environmental behavior (PEB) refers to actions and decisions intended to positively affect the natural environment, such as recycling, conserving energy resources, reducing waste, and supporting sustainable practices.[4] Social value orientation (SVO) is an individual trait that receives significant attention in pro-social behavior research.[5] SVO refers to how individuals prioritize their interests over others in social situations. This study investigates the influence of SVO on PEB. In addition, it aims to determine the difference in the orientation of social values based on gender and whether gender also affects PEB.

Several studies have shown that SVO influences PEB. For example, research consistently shows that individuals with a strong pro-social value orientation are more likely to engage in pro-environmental behaviors, such as recycling and conserving energy resources than those with a more selfish orientation.[6] One study by Bhattacharya[7] found that individuals with a

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strong pro-social value orientation were more likely to engage in pro-environmental efforts. SVO refers to an individual's preference for the distribution of outcomes between themselves and others. It can be classified into three main types: pro-social, pro-self, and individualistic.[8] Research has shown that individuals with a pro-social orientation, who value the well-being of others and the environment, are more likely to engage in pro-environmental behaviors, such as recycling, conserving energy, and supporting environmental policies.[9,10] Pro-social individuals are motivated by altruistic values and a sense of responsibility towards the environment and society. Value-belief norm theory suggests many variables can predict PEB, including SVO.[11,12] Previous research has shown that attitudes towards pro-environmental activities and subjective norms about these behaviors also play an essential role in predicting pro-environmental behaviors.[13] Individuals who prioritize pro-social values are more likely to have stronger pro-environmental beliefs and show a greater willingness to engage in a variety of pro-environmental behaviors compared to those who prioritize individual or selfish values.[14] In addition, research has found a negative correlation between selfish values, traditional conservative values, and PEB.[15,16] Overall, research shows that SVO influences PEB.[14]

It was found that differences in social orientation values based on gender in the context of honesty research show that females are more pro-social than males.[17] There is a similarity in some previous studies; in the context of helpful behavior research, they found that females are more pro-social than males.[18,19] There is also a difference in PEB based on gender; females will be more pro-environmental than males. Supported by some previous studies, females were significantly more engaged in PEB.[20,21] Based on the literature reviewed, it is clear that SVO substantially impacts PEB. There are differences in SVO and PEB based on gender. In this study, the researcher aims to examine the influence of social value orientation on pro-environmental behavior and explain differences based on gender in the context of the environment.

## Methods

This study is a non-experimental quantitative casualty research, which aims to determine the influence of one variable on other variables.[22] The sampling technique used was convenience sampling. This research was conducted from May 2024 to September 2024 at Bandung, B University. The target population of this study consists of active students enrolled in various study programs at B University. Participants were recruited by distributing a survey link presented as a Google Form. This form included informed consent and two measurement tools in the form of questionnaires: the three-dominance social value orientations, which assess individuals' SVO tendencies, and the General Ecological Behavior scale, which evaluates whether individuals tend to engage in pro-environmental or non-pro-environmental behavior. The questionnaire collected demographic information such as age, gender, religion, ethnicity, and study program. The survey link was shared via social media groups and course groups at B University.

The participants were students from various study programs at B University in Bandung, totaling 378 people. Most participants were female (72%) and male (28%).

This study used two questionnaire-measuring tools: the Triple-dominance scale and the General Ecological Behavior measuring tools. A demographic questionnaire was also used to collect participant information, including gender, age, religion, ethnicity, and study program. SVO is measured by the Triple-dominance scale developed by Van Lange in 1997, which consists of 9 items, including three dominant social value orientations: pro-social, individualistic, and competitive. The SVO reliability using the Cronbach's alpha method is 0.883. Each item consists of 3 answer choices that represent the three dominant SVOs. Each participant was asked to choose the most appropriate answer for each item. This measuring tool has undergone an adaptation process using back translation techniques and expert judgment. An example item would be "participants choose between three options: (i) 500 points to themselves (I), 500 points to others (You) (i.e. cooperative choice), (ii) 560 points to themselves, 300 points to others (i.e., individualistic choice), or (iii) 490 points to themselves and 90 points to others (i.e., competitive choice)."

PEB was measured using the General Ecological Behavior (GEB) developed by Florian

G. Kaiser in German and English,[23] which was then adapted into Indonesian with a reliability of 0.739 using the Cronbach's alpha method. The GEB scale is a reliable and valid tool for assessing an individual's commitment to PEB. It has been developed using the Rasch model to ascertain unidimensional and probabilistic measures of PEB.[24] The GEB scale has been used in a variety of contexts, such as assessing adolescent environmental preferences, measuring environmental attitudes and behaviors in children, and capturing ecological lifestyles.[25–27]

The GEB scale has also been used in cross-cultural applications, demonstrating its versatility and applicability in various cultural settings.[28] Only 32 of the 50 GEB scale items are used for this study. It is intended to focus on more relevant and valid ecological behaviors, increasing its sensitivity and alignment with pro-environmental attitudes.[29,30] The GEB scale has option answers ranging from 1 (never) to 5 (always) and 0 (choosing not to answer). GEB-32 has one example: "I am a vegetarian."

Data analysis was carried out with the help of the JASP version 19 program. JASP was used to analyze the descriptive demographics of participants, testing the influence of SVO on PEB and the influence of gender on SVO and PEB. The data from the measurement of SVO is categorical, consisting of the proself (coding: 1) and pro-social (coding: 2) categories. The data from the measurement of the GEB scale is also in the form of categorical data, namely the pro-environmental (coding: 1) and non-pro-environmental (coding: 2) categories. Because the data obtained from the two measuring tools is categorical (nominal), the statistical analysis techniques used are the chi-square test and Fisher's exact test. In addition, an analysis of odd's ratio was also carried out to compare the relative chances of a specific outcome.

## Results

Table 1 shows more female participants (72%) than males (28%). Most participants were 19 years old (38%), with the average age of participants being 19.79 years and the standard deviation being 1.31. Most participants are Muslim (99%) compared to other religions. The most studied programs were psychology (63%) and Sundanese participants (78%), more than other ethnic groups.

**Table 1 Demographic Data**

| Characteristics | n=378 (%) |
|---|---|
| Gender | |
| Female | 273 (72) |
| Male | 105 (28) |
| Age (years) | |
| <18 | 6 (2) |
| 18 | 45 (12) |
| 19 | 143 (38) |
| 20 | 92 (24) |
| 21 | 49 (13) |
| 22 | 28 (7) |
| >22 | 15 (4) |
| Religion | |
| Islam | 375 (99) |
| Protestant | 1 (0.3) |
| Catholic | 1 (0.3) |
| Others | 1 (0.3) |
| Major | |
| Psychology | 238 (63) |
| Faculty of Religion | 81 (21) |
| Law | 31 (8) |
| Economics and Business | 15 (4) |
| Others (Mathematics and Natural Sciences, Engineering, Communication Sciences, and Medicine) | 12 (3) |
| Ethnicity | |
| Sundanese | 296 (78) |
| Javanese | 54 (14) |
| Others (Minang, Bugis and Batak) | 28 (7) |

Table 2 shows that participants are generally more likely to be oriented towards social pro-self values than pro-social, namely 245 people or 65% of participants. It indicates that in the context of social orientation values, more participants tend to be oriented towards their interests rather than the interests of others or the common good; in PEB, more participants are classified as non-pro-environment compared to pro-environmental ones. This condition is also seen in the context of gender. Female participants, compared to males, were more pro-self and not pro-environmental.

Table 3 shows the results of the chi-square test $\chi^2=0.056$ ($p>0.05$), showing no significant difference in SVO based on gender. This means that gender does not influence the orientation of pro-self or pro-social social values.

Table 4 shows the result of Fisher's exact

**Table 2 Contingency**

| Gender | | Social | | Environmental | |
|---|---|---|---|---|---|
| | | Proself | Pro-social | Pro-environmental | Non-pro-environmental |
| Male | Count | 169 | 104 | 8 | 265 |
| | Expected count | 176.944 | 96.056 | 7.222 | 265.778 |
| Female | Count | 76 | 29 | 2 | 103 |
| | Expected count | 68.056 | 36.944 | 2.778 | 102.222 |
| Total | Count | 245 | 133 | 10 | 368 |
| | Expected count | 245 | 133 | 10 | 368 |

**Table 3 Chi-square of Social Value Orientation based on Gender**

| | Value | df | p |
|---|---|---|---|
| $\chi^2$ | 3.649 | 1 | 0.056 |
| n | 378 | | |

**Table 4 Fisher's Exact Test of Pro-environmental Behavior based on Gender**

| | Value | df | p |
|---|---|---|---|
| $\chi^2$ | 0.31 | 1 | 0.578 |
| n | 378 | | |

test p=0.578 (p>0.05), which means there is no gender difference in PEB. So, pro-environmental or non-pro-environmental behavior is not influenced by gender.

Table 5 shows that the majority of participants who are oriented towards pro-self social values show a tendency to behave non-pro-environmentally. As many as 235 participants (95%) are self-oriented. The same results were also found in participants who were oriented towards pro-social social values, where all participants, or 133 who were prosocially oriented, showed non-pro-environmental behavior.

Table 6 shows the results of SVO proven to influence PEB significantly, p=0.017 (p<0.05). It means that the tendency of participants to

**Table 5 Contingency of Chi-Square for Social Value Orientation and Pro-environmental Behavior**

| | | Non-pro-environmental | Pro-environmental | Total |
|---|---|---|---|---|
| Proself | Count | 235 | 10 | 245 |
| | Expected count | 238.519 | 6.481 | 245 |
| Pro-social | Count | 133 | 0 | 133 |
| | Expected count | 129.481 | 3.519 | 133 |
| Total | Count | 368 | 10 | 378 |
| | Expected count | 368 | 10 | 378 |

**Table 6 Fisher's Exact Test for Social Value Orientation Influence on Pro-environmental Behavior**

| | Odds Ratio | 95% CI | | p |
|---|---|---|---|---|
| | | Lower | Upper | |
| Odds ratio | 0.084 | 0.005 | 1.445 | |
| Fisher's exact test | 0 | 0 | 0.805 | 0.017 |

have an SVO has a meaningful influence on their propensity to behave pro-environmental or non-pro-environmental. The odds ratio value of 0.084 (1/0.084=11.90) revealed that pro-social participants were 11.9 times more likely to behave pro-environmental than pro-self value-oriented participants.

## Discussion

This study proves that SVO significantly affects PEB. There is a significant difference between individuals with a tendency to orient pro-social values and those with a tendency to orient pro-social values in choosing non-pro and pro-environmental behaviors. Individuals with a pro-social value orientation were 11.9 times more likely to behave pro-environmental than individuals with a pro-self. Following previous research by Curtin and Jia,[6] pro-social social values-oriented individuals are more likely to engage or tend to behave pro-environmentally. This is supported by the study of Bhattacharya[7] and Zibenberg et al.,[10] which states that individuals with a pro-social value orientation are more likely to engage in PEB. According to the two previous studies, this condition occurs because people oriented to pro-social social values are motivated by altruistic values and a sense of responsibility towards the environment and society.

This study found no influence of gender on SVO or PEB. This finding differs from research conducted by Grosch and Rau,[17] which states that females are more pro-social than males. Similarly, some research has found that females are significantly more likely to engage in PEB.[20,21] For further study, other determinants can be considered to improve PEB for individuals with an SVO of self, such as the demographics of participants who vary in age, religion, study program, university, and ethnicity.

The study had limitations. First is the use of self-report measuring tools, so there is a possibility of bias in the answers. Thus, additional data collection methods are needed using interviews or focus group discussions so that they can provide richer insights into the reasons behind the findings obtained. Second, this study only involves students at one university, so the following research study can include more universities and faculties so that the participants are more varied. Then, this study uses non-probability sampling, so the recommendation for the following research is to use probabilistic sampling. Then, it is necessary to analyze the background of participants other than gender to provide interventions and make more specific policies.

## Conclusion

This study's conclusions are that social value orientation affects pro-environmental behavior and that there is no significant difference between genders in social value orientation tendencies and pro-environmental behavior.

## Conflict of Interest

The author states that there is no conflict of interest. The funders have no role in the research design, data collection, analysis, interpretation, script writing, or decision to publish the results.

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## References

1. Quadrado GP, Dillenburg SR, Goulart ES, Barboza EG. Historical and geological assessment of shoreline changes at an urbanized embayed sandy system in Garopaba, Southern Brazil. Reg Stud Mar Sci. 2021;42:101622.
2. Wakkee I, van der Sijde P, Vaupell C, Ghuman K. The university's role in sustainable development: activating entrepreneurial scholars as agents of change. Technol Forecast Soc Change. 2019;141:195–205.
3. Shafiei A, Maleksaeidi H. Pro-environmental behavior of university students: application of protection motivation theory. Glob Ecol Conserv. 2020;22:e00908.
4. Kurisu K. Pro-environmental behaviors.

Tokyo: Springer Japan; 2015.
5. Bakker DM, Dijkstra J. Comparing the slider measure of social value orientation with its main alternatives. Soc Psychol Q. 2021;84(3):235–45.
6. Curtin D, Jia F. Revisiting social value orientations and environmental attitude–identity–intention in decomposed games. Int J Environ Res Public Health. 2022;19(12):6961.
7. Bhattacharya H. Do pro-social students care more for the environment? Int J Sustain High Educ. 2019;20(4):761–83.
8. Iwai T, Tavares GM. When prosocial motives matter most: the interactive effects of social value orientation, message framing, and helping costs on helping behavior. J Behav Decis Mak. 2024;37(2):e2384.
9. Lee YK, Kim S, Kim MS, Choi JG. Antecedents and interrelationships of three types of pro-environmental behavior. J Bus Res. 2014;67(10):2097–105.
10. Zibenberg A, Greenspan I, Katz-Gerro T, Handy F. Environmental behavior among Russian youth: The role of self-direction and environmental concern. Environ Manag. 2018;62(2):295–304.
11. Liobikienė G, Liobikas J, Brizga J, Juknys R. Materialistic values impact on pro-environmental behavior: the case of transition country as Lithuania. J Clean Prod. 2020;244:118859.
12. Palupi T, Sawitri DR. The importance of pro-environmental behavior in adolescent. E3S Web Conf. 2018;31:09031.
13. Schultz PW, Nolan JM, Cialdini RB, Goldstein NJ, Griskevicius V. The constructive, destructive, and reconstructive power of social norms: reprise. Perspect Psychol Sci. 2018;13(2):249–54.
14. Marcus K. The fundamental role of large-scale trust building in natural resource management. Environ Values. 2016;25(3):259–86.
15. Agissova F, Sautkina E. The role of personal and political values in predicting environmental attitudes and pro-environmental behavior in Kazakhstan. Front Psychol. 2020;11:584292.
16. De Groot JIM, Thøgersen J. Values and pro-environmental behaviour. In: Steg L, De Groot JIM, editors. Environmental psychology: an introduction. 2nd Edition. Hoboken: John Wiley & Sons Ltd; 2019. p. 167–178.
17. Grosch K, Rau HA. Gender differences in honesty: the role of social value orientation. 2017. J Econ Psychol. 2017;62:258–67.
18. artínez-Gregorio S, Tomás JM, Oliver A. A psychometric study of the prosocial behavior scale: differential item functioning by gender. Behav Sci (Basel). 2023;13(3):259.
19. spinosa MP, Kovářík J. Prosocial behavior and gender. Front Behav Neurosci. 2015;9:88.
20. ien YH, Huang J. Gender differences in pro-environmental behavioral intentions, environmental values, tolerance of environmental protection cost, and confidence in citizen participation in environmental policies during the COVID-19 pandemic in Taiwan. Pol J Environ Stud. 2023;32(5):4813–23.
21. Li Y, Wang B, Saechang O. Is female a more pro-environmental gender? Evidence from China. Int J Environ Res Public Health. 2022;19(13):8002.
22. Sari AQ, Sukestiyarno YL, Agoestanto A. Batasan prasyarat uji normalitas dan uji homogenitas pada model regresi linear. UJM. 2017;6(2):168–77.
23. Kaiser FG. GEB-50. General Ecological Behavior scale. Trier, Germany: Leibniz Institute for Psychology; 2020 [cited 2024 July 20]. Available from: https://www.testarchiv.eu/en/test/9004402.
24. Pronello C, Gaborieau JB, Rappazzo V, Operti V. Case study on effects of the mandatory validation on bus commercial speed. EJTIR. 2019;19(1):43–59.
25. Bogner FX, Suarez BR. Environmental preferences of adolescents within a low ecological footprint country. Front Psychol. 2022;13:894382.
26. Balundė A, Perlaviciute G, Truskauskaitė-Kunevičienė I. Sustainability in youth: environmental considerations in adolescence and their relationship to pro-environmental behavior. Front Psychol. 2020;11:582920.
27. Arnold O, Kibbe A, Hartig T, Kaiser FG. Capturing the environmental impact of individual lifestyles: evidence of the criterion validity of the general ecological behavior scale. Environ Behav. 2018;50(3):350–72.
28. Kovács B, Carroll GR, Lehman DW. Authenticity and consumer value ratings:

empirical tests from the restaurant domain. Organ Sci. 2013;25(2):458–78.
29. Otto S, Kröhne U, Richter D. The dominance of introspective measures and what this implies: the example of environmental attitude. PLoS One. 2018;13(2):e0192907.
30. Markle GL. Pro-environmental behavior: does it matter how it's measured? Development and validation of the pro-environmental behavior scale (PEBS). Hum Ecol. 2013;41(6):905–14.

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**RESEARCH ARTICLE**

# Autism Spectrum Disorder: a Two-center Evaluation of Pharmacological Intervention and Behavioral Therapies on Core Symptoms

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**Abstract**

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by chronic deficits in social communication and interaction, with sensory processing abnormalities affecting over 90% of individuals across different sensory areas. This study investigated the combined effects of aripiprazole and behavioral therapy (BT) on core symptoms of ASD in children aged 6–10. Utilizing the considerable neuroplasticity still present at this age, we hypothesized that this combined approach might yield superior outcomes compared to BT alone. The 12-week randomized, double-blind, placebo-controlled trial was conducted from February 2023 to January 2024 at two sites of Child Development Centers in Bandung city, involving 51 participants (22 intervention, 29 placebo). The intervention group received aripiprazole and BT, while the placebo group received saccharum lactis and BT. Both groups underwent BT comprising applied behavioral analysis (ABA) and discrete trial training (DTT). The Childhood Autism Rating Scale (CARS) assessed treatment effects. The independent 2-sample t-tests and Mann-Whitney tests showed no significant differences in overall CARS scores between groups. However, the analysis revealed significant improvements in three subcategories: VII (visual response, p=0.021), IX (taste-smell-touch response, p=0.035), and X (fear or nervousness, p=0.043). These findings suggest that the combined approach may enhance sensory processing and emotional regulation in children with ASD. The study highlights the potential benefits of a multimodal approach to ASD treatment, combining targeted pharmacological intervention with behavioral therapies. However, limitations such as study duration and sample size warrant further research to optimize treatment strategies for individuals across the autism spectrum.

**Keywords:** Aripiprazole, autism spectrum disorder, behavioral therapy, Childhood Autism Rating Scale, sensory processing

## Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by persistent impairments in social communication and interaction, along with restricted and repetitive patterns of behaviors, interests, or activities.[1] The global prevalence of ASD is estimated at 6 cases per 1,000 individuals in Southeast Asian countries, with a significant male predominance.[2] Children with ASD often experience additional challenges, including aggressive behaviors, self-injurious behaviors, tantrums, and irritability.[3]

Diagnosis of ASD involves a multifaceted approach, incorporating behavioral observation, parental interviews, and standardized assessment tools, with updated criteria provided by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).[1] However, the heterogeneous presentation in ASD poses significant challenges in assessment and diagnosis. The Childhood Autism Rating Scale (CARS) is a widely utilized instrument for differentiating between mild-to-moderate and severe ASD in children aged two and above.[4]

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Sensory processing abnormalities are a core feature of ASD, affecting over 90% of individuals across multiple sensory modalities (visual, auditory, tactile, gustatory, and olfactory).[5] These atypical sensory responses manifest as hypo-responsiveness, hyper-responsiveness, and sensory-seeking behaviors, with some researchers proposing an additional pattern of enhanced perception. These abnormalities can significantly impact daily functioning, academic performance, and social interactions. Studies using tools like the Sensory Processing Measure, Second Edition (SPM-2) have shown that children with ASD exhibit higher scores across all subscales, indicating worse sensory processing, praxis, and social participation in daily activities than typically developing peers.[6,7]

While behavioral interventions for ASD symptoms like hyperactivity and irritability exist, effective treatments for sensory abnormalities remain limited.[8,9] Recent research has focused on aripiprazole, a novel antipsychotic with a unique pharmacodynamic profile.[9] Aripiprazole acts as a partial agonist at dopamine D2 and serotonin 5-HT1A receptors and an antagonist at 5-HT2A receptors. FDA-approved for treating ASD-associated irritability in children aged 6–17, ongoing studies are evaluating its efficacy in managing sensory processing abnormalities in ASD.[9] This research may provide new therapeutic options for addressing the complex sensory issues inherent to ASD.

As our understanding of sensory processing in ASD continues to evolve, it is crucial to develop targeted interventions and support strategies to enhance the overall functioning and quality of life for children with ASD. This study may provide insights into the efficacy of aripiprazole and behavioral therapy (BT) in managing the complex core symptoms associated with ASD.

## Methods

This randomized, double-blind, fixed-dose, placebo-controlled study was conducted at two sites (Melinda 2 Child Developmental Center & Indigrow-Child Development Center) in Bandung city, Indonesia, between February 2023 and January 2024. The ethics committee approved the study from the Research Ethics Committee of Universitas Padjadjaran with letter number 988/UN6/KEP/EC/2022.

The study focused on a group of outpatients between the ages of 6–10 who had been diagnosed with autistic disorder as their primary condition according to DSM-V criteria[1] and had not previously undergone any pharmacological treatment. The study excluded participants who had been diagnosed with Asperger syndrome, pervasive developmental disorder-not otherwise specified, Rett syndrome, childhood disintegrative disorder, or intellectual disability.[3]

An experienced pediatric neurologist validated the diagnosis using the CARS Indonesian version (sensitivity of 85.2%, accuracy of 85.7%, and internal consistency of 0.819).[10] CARS scores range from 15 to 60, with a 30 or above indicating autism.[4] Following 12 weeks of medication and behavioral therapy, subjects underwent a repeat CARS assessment to evaluate treatment effects.

Eligible participants were randomly assigned using GraphPad randomizer software by Dotmatics, with results converted into sequentially numbered opaque sealed envelope (SNOSE) and further randomized by a third-party research assistant. Participants were divided into an intervention group (aripiprazole+BT) and a placebo group (saccharum lactis+BT). The intervention group received Abilify Discmelt® orally disintegrating tablets (10 mg, Otsuka Indonesia),[11] while the placebo group received saccharum lactis powder (DFE Pharma GmbH & Co. KG, Germany).[12] Both treatments were given in powder form, and the intervention dosage schedule was 10 mg/day (week 1), 5 mg/day (weeks 2–5), and 10 mg/day (weeks 6–12).

Alongside pharmacotherapy, participants underwent intensive BT comprising applied behavioral analysis (ABA) and discrete trial training (DTT) for 12 weeks (5 sessions/week, 60 total, 75% minimum attendance).[13] Each session comprised 50 minutes of therapy and 10 minutes of parent counseling conducted by certified therapists (>10 years experience). Following the initial assessment, therapists documented daily progress notes, monitored medication adherence, and facilitated parental logbook maintenance for outcome evaluation.

The data collected were analyzed using the Statistical Package for Social Sciences (SPSS) version 22 for Windows. Independent 2-sample t-tests were applied when the data followed a normal distribution, while the Mann-Whitney U test was used for non-normally distributed data.

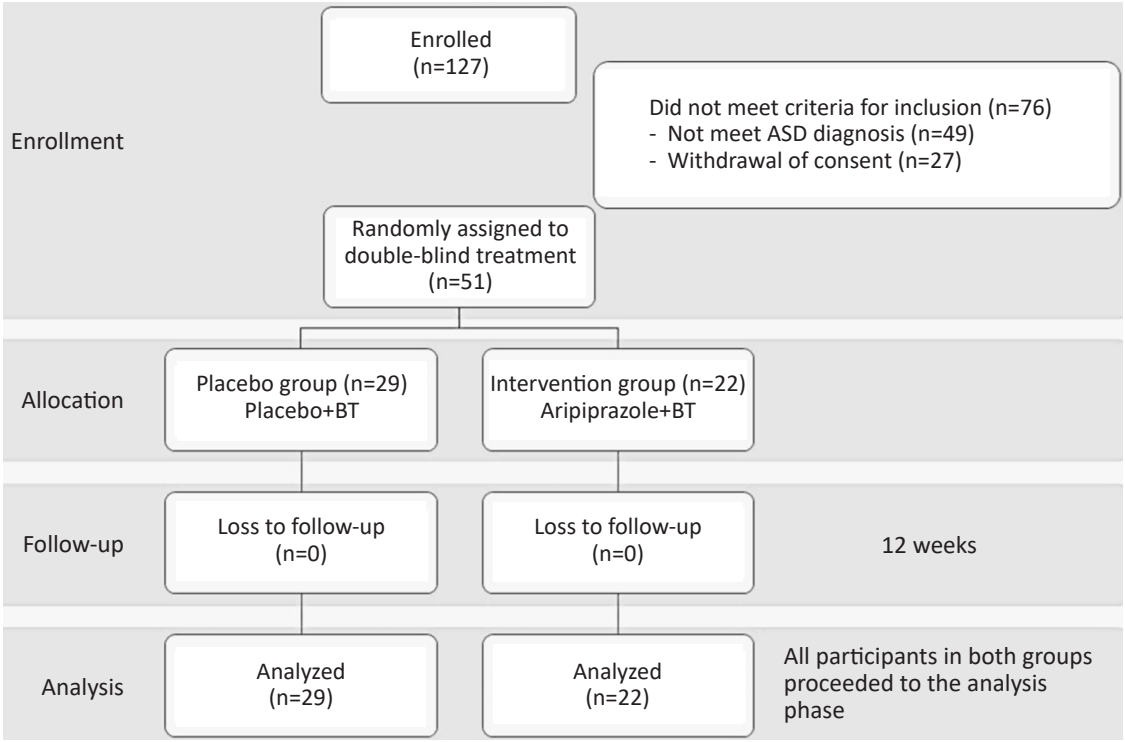


**Figure Participant Flow**

These tests aimed to assess whether there was a significant improvement of core symptoms in ASD between the placebo and intervention groups, with a significance threshold set at $p<0.05$.

## Results

This study included 51 participants (22 intervention, 29 placebo) with similar mean ages (intervention: 6.82±0.39 years, placebo: 6.60±0.37 years). Participants had diverse educational backgrounds and varied gender distribution (intervention: 18M/4F; placebo: 18M/11F) as shown in Table 1.

Table 2 shows that baseline and final total CARS scores showed no significant differences between groups. However, further analysis in Table 3 revealed significant differences ($p<0.05$) in three CARS sub-categories after 12 weeks of treatment: VII (visual response), IX (taste, smell,

**Table 1 Contingency of Chi-Square for Social Value Orientation and Pro-environmental Behavior**

| Characteristics | Placebo Group n=29 | Intervention Group n=22 |
|---|---|---|
| Age (years) | | |
| Mean (SD) | 6.60 (±0.37) | 6.82 (±0.39) |
| Gender | | |
| Boys | 18 | 18 |
| Girls | 11 | 4 |
| School placement | | |
| General school | 7 | 13 |
| Special school | 6 | 4 |
| Homeschooling | 2 | 0 |
| Do not attend | 14 | 5 |

**Table 2 Clinical Measure of CARS**

| Clinical Measure | Placebo Group n=29 | Intervention Group n=22 | p |
|---|---|---|---|
| Total score CARS (mean±SD) | | | |
| Baseline | 40.00 (±1.21) | 42.16 (±0.97) | 0.310 |
| End of treatment | 35.00 (±1.32) | 37.62 (±0.88) | 0.054 |

Note: t-test independent

**Table 3 Profile of CARS Sub-category at the End of Treatment**

| Sub-Category CARS (Mean±SD) | Placebo Group n=29 | Intervention Group n=22 | p |
|---|---|---|---|
| CARS I (Relationship with people) | 2.58 (±0.116) | 2.29 (±0.121) | 0.137 |
| CARS II (Imitation) | 2.53 (±0.124) | 2.38 (±0.135) | 0.582 |
| CARS III (Emotional response) | 2.46 (±0.092) | 2.25 (±0.108) | 0.182 |
| CARS IV (Body use) | 2.39 (±0.091) | 2.34 (±0.129) | 0.574 |
| CARS V (Object use) | 2.43 (±0.101) | 2.23 (±0.138) | 0.196 |
| CARS VI (Adaptation to change) | 2.39 (±0.10) | 2.34 (±0.124) | 0.720 |
| CARS VII (Visual response) | 2.43 (±0.90) | 2.7 (±0.10) | 0.021* |
| CARS VIII (Auditory response) | 2.17 (±0.090) | 2.07 (±0.10) | 0.520 |
| CARS IX (Taste, smell, and touch response) | 2.24 (±0.128) | 1.91 (±0.153) | 0.035* |
| CARS X (Fear or nervousness) | 2.39 (±0.094) | 2.14 (±0.10) | 0.043* |
| CARS XI (Verbal communication) | 2.89 (±0.134) | 2.86 (±0.128) | 0.745 |
| CARS XII (Non-verbal communication) | 2.60 (±0.109) | 2.43 (±0.120) | 0.487 |
| CARS XIII (Activity level) | 2.45 (±0.071) | 2.32 (±0.101) | 0.386 |
| CARS XIV (Intellectual inconsistency) | 2.72 (±0.130) | 2.70 (±0.149) | 0.853 |
| CARS XV (General impression) | 2.89 (±0.112) | 2.66 (± 0.120) | 0.174 |

Note: Mann-Whitney U test, *significance p<0.05

and touch response), and X (fear or nervousness). These findings suggest the treatment significantly impacted specific aspects of autism core symptoms in both groups.

## Discussion

This comprehensive study investigates the combined effects of pharmacological treatment (specifically aripiprazole) and behavioral interventions on core symptoms of ASD, utilizing the CARS to assess various aspects of the disorder, including social/communication skills, stereotyped behavior, sensory abnormalities, and emotional regulation.[4,10]

While the overall CARS scores did not show statistically significant differences between groups at the 12-week intervention, a change of at least 4.5 points in CARS score is considered a benchmark for successful intervention. However, the clinical relevance of minimal changes can vary between individuals.[14] A more detailed analysis revealed notable improvements in three specific CARS sub-categories: VII (visual response), IX (taste-smell-touch response), and X (fear or nervousness). These findings suggest potential advancements in sensory processing and emotional regulation, two domains frequently challenging for individuals with ASD.

Aripiprazole, a second-generation (atypical) antipsychotic, has been well-documented for its efficacy in reducing disruptive behaviors in individuals with ASD, leading to its FDA approval for treating irritability.[15,16] A cohort study by Marcus et al.[17] demonstrated that aripiprazole (2–15 mg/day) over one year significantly reduced Aberrant Behavior Checklist-Irritability (ABC-I) scores by −12.9 compared to −5 in the placebo group, with improvements observed as early as the first week at 2 mg/day. A retrospective study by Fung et al.[18] found that aripiprazole improved sensory symptoms in autistic children and

adolescents, particularly inattention, auditory processing, and visual input affecting emotional responses and activity levels.

The study underscores the potential advantages of a multimodal approach to ASD treatment, combining targeted pharmacological interventions with BT. Intensive behavioral intervention (IBI) alone showed substantial decreases in aggressive behavior but was more effective when paired with antipsychotic medication.[19,20] Recent studies in mice have provided a neurobiological basis for these observations, showing that chronic administration of risperidone or aripiprazole improves social interaction deficits and recognition memory impairment, with reductions observed in dendritic spine density in the prefrontal cortex and hippocampus.[21,22]

Strong connections were found between sensory processing and communication abilities in ASD children, with overall sensory scores correlating positively with most communication skills. Interestingly, all patterns of sensory processing showed significant negative correlations with anxiety subsets in ASD children, suggesting that children with more pronounced sensory processing difficulties tended to experience lower levels of anxiety. Social relationship scores in ASD children correlated negatively with all anxiety subsets, implying that improving social relationships may help reduce anxiety and vice versa.[23]

ABA and DTT were identified as two of 27 evidence-based practices for individuals with autism. The study suggests that BT for autism should target core symptoms, address co-occurring issues, be adapted to the individual's cognitive and developmental level, incorporate visual supports and structured teaching, and focus on generalizing skills to natural environments.[24]

The prognosis for individuals with ASD is influenced by several factors, including the child's age when therapy is first given, the intensity of therapy, therapy techniques, parental involvement, and the child's characteristics (such as intelligence level, language capacity, and behavioral problems). Early intervention has significantly improved in several domains, including communication, social-emotional functioning, adaptive behavior, and physical development.[25]

Early IBI is recommended for preschool to early school children with autism, with a minimum of 20–40 hours per week.[8] Behavioral therapy for children with ASD should be tailored to different developmental stages throughout life. In early childhood, interventions focus on language acquisition, play skills, joint attention, and effective communication strategies through intensive behavioral and educational interventions, especially ABA. During childhood and middle adolescence, the focus shifts to continuing skill development, including social skills, peer relationships, and maximizing academic support. For older adolescents and young adults, developing vocational and adaptive life skills becomes crucial to maximize opportunities for independence and support the transition as caregivers age.[26]

Despite its promising findings, the study has several limitations, including suboptimal duration of behavioral therapy, variations in subject compliance and parental involvement, the influence of underlying medical conditions on treatment adherence, genetic variations affecting treatment responses, relatively short study duration, potentially limited sample size, and lack of control for concurrent interventions. These limitations highlight the complex nature of ASD treatment and the need for individualized approaches.

Future research should focus on elucidating underlying processes, developing integrated therapies for sensory processing difficulties, communication challenges, and anxiety in ASD, optimizing BT for individuals across the autism spectrum, evaluating long-term outcomes, and combining BT with other evidence-based practices. This comprehensive approach to ASD treatment, combining pharmacological interventions with behavioral therapies, holds promise for addressing both behavioral and sensory symptoms in ASD, potentially leading to more effective and personalized treatment strategies.

Understanding the nuanced relationships between cognitive functioning and sensory processing in ASD is essential for developing targeted and individualized interventions. By considering cognitive abilities and sensory processing when developing support strategies, clinicians can create more effective treatments that address specific sensory challenges based on an individual's cognitive profile.[27] This approach

has the potential to significantly improve the quality of life for those on the autism spectrum across different developmental stages and cognitive levels.

## Conclusions

In conclusion, combined pharmacological and behavioral therapy effectively reduced core symptom complexity in children with ASD. Significant improvements in total CARS scores, especially in subcategories VII (visual response), IX (taste, smell, and touch response), and X (fear or nervousness), indicate enhanced sensory processing and emotional regulation. These early positive changes suggest this combined approach is a promising treatment strategy, potentially improving overall functioning and quality of life for children with ASD.

## Conflict of Interest

No conflicts of interest occur in this study.

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## References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th Edition. Arlington: American Psychiatric Publishing; 2013.
2. Shrestha M, Basukala S, Thapa N, Shrestha O, Basnet M, Shrestha K, et al. Prevalence of autism spectrum disorder among children in Southeast Asia from 2002 to 2022: an updated systematic review and meta-analysis. Health Sci Rep. 2024;7(4):e2005.
3. Rosen NE, Lord C, Volkmar FR. The diagnosis of autism: from Kanner to DSM-III to DSM-5 and beyond. J Autism Dev Disord. 2021;51(12):4253–70.
4. Moulton E, Bradbury K, Barton M, Fein D. Factor analysis of the Childhood Autism Rating Scale in a sample of two year olds with an autism spectrum disorder. J Autism Dev Disord. 2019;49(7):2733–46.
5. Robertson CE, Baron-Cohen S. Sensory perception in autism. Nat Rev Neurosci. 2017;18(11):671–84.
6. Patil O, Kaple M. Sensory processing differences in individuals with autism spectrum disorder: a narrative review of underlying mechanisms and sensory-based interventions. Cureus. 2023;15(10):e48020.
7. Narzisi A, Fabbri-Destro M, Crifaci G, Scatigna S, Maugeri F, Berloffa S, et al. Sensory profiles in school-aged children with autism spectrum disorder: a descriptive study using the Sensory Processing Measure-2 (SPM-2). J Clin Med. 2022;11(6):1668.
8. Reichow B, Hume K, Barton EE, Boyd BA. Early intensive behavioral intervention (EIBI) for young children with autism spectrum disorders (ASD). Cochrane Database Syst Rev. 2018;5(5):CD009260.
9. VanDerwall R, Rotta K, Ehrhardt K, Poling A. Using aripiprazole to benefit people with autism spectrum disorder: a critical appraisal. Adv Neurodev Disord. 2021;5(1):1–10.
10. Sari SH. Childhood autism: the internal consistency Childhood Autism Rating Scale for use in Indonesia and descriptive study of autism clinical variance [undergraduate thesis]. Semarang: Universitas Diponegoro; 2009. Available from: http://eprints.undip.ac.id/7487/1/Stefani_harum_sari.pdf.
11. Otsuka Pharmaceutical Company. Highlights of prescribing information [Internet]. Tokyo: Otsuka Pharmaceutical Company; 2016 [cited 2024 September 20]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021436s041%2C021713s032%2C021729s024%2C021866s026lbl.pdf.
12. Chand R, Sharma A, Chaudhary S. Saccharum lactis a placebo or homoeopathic medicine: a mystery vehicle. Homoeopath Herit. 2024;50(4):14–6.
13. Slocum TA, Detrich R, Wilczynski SM, Spencer TD, Lewis T, Wolfe K. The evidence-based practice of applied behavior analysis. Behav Anal. 2014;37(1):41–56.
14. Jurek L, Baltazar M, Gulati S, Novakovic N, Núñez M, Oakley J, et al. Response (minimum clinically relevant change) in ASD symptoms after an intervention according to CARS-2: consensus from an expert elicitation procedure. Eur Child Adolesc Psychiatry. 2022;31(8):1–10.
15. Ichikawa H, Mikami K, Okada T, Yamashita

Y, Ishizaki Y, Tomoda A, et al. Aripiprazole in the treatment of irritability in children and adolescents with autism spectrum disorder in Japan: a randomized, double-blind, placebo-controlled study. Child Psychiatry Hum Dev. 2017;48(5):796–806.
16. Rizzo R, Pavone P. Aripiprazole for the treatment of irritability and aggression in children and adolescents affected by autism spectrum disorders. Expert Rev Neurother. 2016;16(8):867–74.
17. Marcus RN, Owen R, Manos G, Mankoski R, Kamen L, McQuade RD, et al. Aripiprazole in the treatment of irritability in pediatric patients (aged 6–17 years) with autistic disorder: results from a 52-week, open-label study. J Child Adolesc Psychopharmacol. 2011;21(3):229–36.
18. Fung LK, Chahal L, Libove RA, Bivas R, Hardan AY. A retrospective review of the effectiveness of aripiprazole in the treatment of sensory abnormalities in autism. J Child Adolesc Psychopharmacol. 2012;22(3):245–8.
19. Frazier TW, Youngstrom EA, Haycook T, Sinoff A, Dimitriou F, Knapp J, et al. Effectiveness of medication combined with intensive behavioral intervention for reducing aggression in youth with autism spectrum disorder. J Child Adolesc Psychopharmacol. 2010;20(3):167–77.
20. Chung KM, Chung E, Lee H. Behavioral interventions for autism spectrum disorder: a brief review and guidelines with a specific focus on applied behavior analysis. Soa Chongsonyon Chongsin Uihak. 2024;35(1):29–38.
21. Hara Y, Ago Y, Taruta A, Hasebe S, Kawase H, Tanabe W, et al. Risperidone and aripiprazole alleviate prenatal valproic acid-induced abnormalities in behaviors and dendritic spine density in mice. Psychopharmacology (Berl). 2017;234(21):3217–28.
22. Alsayouf HA, Talo H, Biddappa ML, De Los Reyes E. Risperidone or aripiprazole can resolve autism core signs and symptoms in young children: case study. Children (Basel). 2021;8(5):318.
23. Khaledi H, Aghaz A, Mohammadi A, Dadgar H, Meftahi GH. The relationship between communication skills, sensory difficulties, and anxiety in children with autism spectrum disorder. Middle East Curr Psychiatry. 2022; 29:69.
24. Wong C, Odom SL, Hume KA, Cox AW, Fettig A, Kucharczyk S, et al. Evidence-based practices for children, youth, and young adults with autism spectrum disorder: a comprehensive review. J Autism Dev Disord. 2015;45(7):1951–66.
25. Tiura M, Kim J, Detmers D, Baldi H. Predictors of longitudinal ABA treatment outcomes for children with autism: a growth curve analysis. Res Dev Disabil. 2017;70:185–97.
26. Politte LC, Howe Y, Nowinski L, Palumbo M, McDougle CJ. Evidence-based treatments for autism spectrum disorder. Curr Treat Options Psychiatry. 2015;2:38–56.
27. Odermatt SD, Möhring W, Grieder S, Grob A. Cognitive and developmental functions in autistic and non-autistic children and adolescents: evidence from the intelligence and development scales–2. J Intell. 2022; 10(4):112.

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**RESEARCH ARTICLE**

# Correlation between Length-to-width Ratio of Gallbladder and Gamma-glutamyl Transferase Value in Biliary Atresia

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**Abstract**

Biliary atresia represents one of the most prevalent etiologies for neonatal cholestasis. Unmanaged biliary atresia can be fatal. Ultrasonography is the primary diagnostic test because it's accurate, cost-effective, and available. Various ultrasound findings can assist in diagnosing biliary atresia; the length-to-width ratio of the gallbladder is a particularly advantageous method, offering a short examination time, objectivity, and ease of use, with an accuracy rate of 78.9%. In cases with unconventional ultrasound findings, gamma-glutamyl transferase is believed to be able to complete the diagnostic process with an accuracy rate of up to 80%. The optimal cut-off value differs between studies, making it challenging to use as a benchmark for biliary atresia detection. In this study, researchers aim to further investigate the relationship between length-to-width ratio and gamma-glutamyl transferase in cases of biliary atresia, compared to the liver biopsy results in these patients and the optimal cut-off. This study employed an observational analytic approach with a retrospective design. The sample population consisted of all patients with neonatal cholestasis who underwent laboratory and ultrasonographic examinations at Dr. Soetomo Academic General Hospital Surabaya between 2019 and 2023. The study population comprised 82 patients. A significant relationship (p-value<0.001) was observed between the length-to-width ratio of the gallbladder and biliary atresia, as well as between gamma-glutamyl transferase and biliary atresia (area under the curve: 0.7–0.8). However, the analysis between the length-to-width ratio of the gallbladder and the value of gamma-glutamyl transferase showed p-value=0.066, which means no significant relationship was observed between the length-to-width ratio and gamma-glutamyl transferase.

**Keywords:** Biliary atresia, gamma-glutamyl transferase, length-to-width ratio, ultrasound

## Introduction

The reported incidence of biliary atresia worldwide varies from 1 in 8,000 to 19,000 live births, with females outnumbering males (1.4:1), with the highest incidence in East Asia. The reason for this high incidence remains unexplained. While epidemiological data on biliary atresia in Indonesia is not yet widely available, it is the most common cause of obstructive cholestasis identified in the first three months of life. Furthermore, in advanced stages, it can contribute to neonatal mortality. Consequently, biliary atresia is often considered physiological neonatal jaundice in its early phase.[1–3]

The diagnosis of biliary atresia is often based on clinical judgment, as there is no specific examination. Laboratory and imaging findings frequently overlap with other etiologies of cholestasis. In selecting imaging modalities, some studies state that ultrasonography (USG) is the modality of choice to diagnose biliary atresia and the most common non-invasive examination performed in preparation for further action.[4,5]

A study conducted in China obtained a comparison of triangular cord signs with gallbladder classification in biliary atresia patients; the accuracy of gallbladder classification was 10% higher when compared to triangular cord signs. Meanwhile, using the gallbladder's length-to-width ratio (LTWR), a high diagnostic value was obtained with an area under the curve of 0.844.[6] In contrast to the previous study conducted at Dr. Soetomo Academic General Hospital, there was no significant relationship between gallbladder wall echogenicity or gallbladder contractility on

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liver biopsy results.[7]

Several studies have been conducted to detect biliary atresia early, but many aspects still need to be studied. In practice, ultrasound examination in children requires conducive conditions and a short implementation time. A multidisciplinary approach is expected to make the diagnosis more accurate.[8]

Another indicator often used is the gamma-glutamyl transferase (GGT) value, which is believed to significantly improve the accuracy of biliary atresia diagnosis. In a previous study, a sensitivity of 85% with a specificity of 88% was obtained,[9] and in another study, the sensitivity was 86.7% and specificity 65%.[10] However, the optimal cut-off value differed from each study, making it difficult to adapt as a benchmark for early detection of biliary atresia.

Limited literature explicitly mentions the relationship between gallbladder LTWR and GGT values in patients with biliary atresia. The researcher is interested in continuing research on this relationship, which will be compared with the liver biopsy results in these patients.

This analysis is expected to provide new insights into the correlation of LTWR and GGT values in patients with biliary atresia. Additionally, it furnishes data regarding the newly established cut-off value of length and the length-to-width ratio for biliary atresia. With a better understanding of this relationship, early detection can be done appropriately so that the treatment and management of patients with biliary atresia can be further improved and more effective radiology services can be provided. This study aims to investigate further the relationship between length-to-width ratio and gamma-glutamyl transferase in cases of biliary atresia, comparing the liver biopsy results in these patients and the optimal cut-off.

## Methods

This study was an observational analytic study with a retrospective approach. The sample population consisted of patients with neonatal cholestasis who underwent laboratory and ultrasound examinations at Dr. Soetomo Academic General Hospital Surabaya. The Health Research Ethics Committee of Dr. Soetomo Academic General Hospital has approved the procedure with a letter of exemption registration number 1670/LOE/301.4.2/V/2024. Data were collected from all patients diagnosed with cholestasis based on clinical patients who had undergone laboratory examination, ultrasonography, and liver biopsy from January 2019 to December 2023 with accessible medical record data. Laboratory results must have a difference of less than 30 days from the ultrasound examination to reduce the bias factor in this study. The final diagnosis was obtained from histopathological results or final diagnosis data based on clinician agreement seen from medical record data.

Researchers identified patients with neonatal cholestasis who had undergone ultrasound examination and then re-evaluated the ultrasound findings by researchers under the supervision and guidance of 2 pediatric radiology consultants and compared them with laboratory and histopathological results of liver biopsy from medical record data. The inter-observer agreement was evaluated with Cohen's kappa coefficient test, with a significance level of $p<0.05$. An agreement value of 0.8–0.9 was obtained. Subsequently, the between variables were compared to identify the significance value. The results were considered significant if the p-value was <0.05 with a 95% confidence interval (CI). The data were analyzed using SPSS 23 statistical software.

The data obtained were then analyzed and presented as research results. The cut-off used in this study, LTWR >5.2 and GGT value >188, follows the prior study conducted by Wang et al.[6] and Zhou and Zhou[11] in China. Relevant guidelines and regulations are carried out in all methods.

## Results

The sample was 82 patients, with 49 males (59.8%) and 33 females (38.4%). The final diagnosis of biliary atresia was more prevalent in males, with 23 males (56.1%) and 18 females (43.9%). The age group of ≤30 days represented the smallest sample size, comprising only two individuals (2.4%), whereas the age group of ≥121 days had the largest sample size, with 28 individuals (34.2%). The age group of ≥121 days also had the highest prevalence of biliary atresia, accounting for 39% of the cases. The distribution of samples according to age group is presented in Table 1.

**Table 1 Age Distribution**

| Range of Age (Days) | Diagnosis | | Total n=82 (%) |
|---|---|---|---|
| | Biliary Atresia n=41 (%) | Non-biliary Atresia n=41 (%) | |
| ≤30 | 0 (0) | 2 (4.9) | 2 (2.4) |
| 31–60 | 8 (19.5) | 10 (24.4) | 18 (22.0) |
| 61–90 | 11 (26.9) | 12 (29.2) | 23 (28.0) |
| 91–120 | 6 (14.6) | 5 (12.2) | 11 (13.4) |
| ≥121 | 16 (39.0) | 12 (29.3) | 28 (34.2) |

**Table 2 LTWR and GGT Data**

| | Abnormality | Median | Minimum | Maximum |
|---|---|---|---|---|
| LTWR | <0.05 | 4.345 | 1.17 | 21.31 |
| GGT | <0.05 | 369.550 | 12.80 | 3,964.20 |

Length-to-width ratio (LTWR) data were obtained from the results of reviewing the abdominal ultrasonography results performed by researchers under the supervision of 2 pediatric consultant radiology specialists with the results of the agreement test (kappa) on significant gallbladder length-to-width ratio data with a strength >0.9 and abnormal data distribution so that the data characteristics were grouped by median (4.34), minimum (1.17) and maximum (21.31). Gamma-glutamyl transferase (GGT) data was obtained from medical records with abnormal data distribution results, so the data characteristics are grouped by median (369.55), minimum (12.8), and maximum (3,964.20). Data on LTWR and GGT is presented in Table 2.

Data analysis was performed by examining the relationship of each variable to biliary atresia, calculations using 95% confidence intervals and p-values were considered significant if $p<0.05$, LTWR and GGT have a p-value<0.001 which means there is a very substantial relationship with biliary atresia. Then, the ROC curve was made, the area under the curve was measured, and the

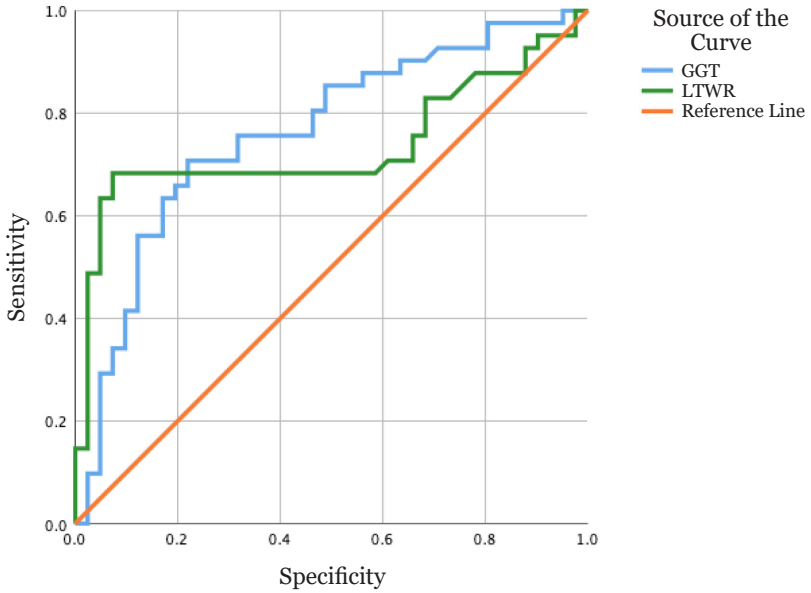


**Figure 1 ROC Curve**

study cut off with a 95% confidence interval, as presented in Figure 1. The cut-off value of length to width ratio of gallbladder was >4.75, and the cut-off value of GGT was >390.

Table 3 illustrates that diagnostic tests conducted for the two variables, compared with histopathological results, demonstrated that LTWR of the gallbladder exhibited the highest accuracy among the variables, at 78.04%. Upon applying the research cut-off (>4.75), this accuracy value increased to 80.48%.

The results of the analysis of the relationship between the gallbladder's LTWR and the value of GGT in biliary atresia showed a value of p=0.066 (p>0.05) with a correlation coefficient of 0.204, which means there is no significant relationship between the LTWR and the value of GGT (Table 4).

## Discussion

Females are more common in cases of biliary atresia, and in this study, biliary atresia data were more dominant in men (56.1%) than in women (43.9%). This aligns with previous research conducted at Dr. Soetomo Academic General Hospital, where there was more male sample than female.[7] However, the previous literature did not explain the relationship between gender and biliary atresia. Epidemiologically, biliary atresia can be found in both males and females, with a probability percentage that is not very different. Still, sometimes, there is a gap in the prevalence of men and women; in general, of all congenital abnormalities that have been reported, men dominate the majority. In biliary atresia,

**Table 3 AUC between Literature and This Study Cut-off**

| | AUC | Significance | Literature Cut-off | Study Cut-off |
|---|---|---|---|---|
| LTWR | 0.735 | <0.001 | ≥5.2 | >4.75 |
| GGT | 0.761 | <0.001 | >188 | >390 |

**Table 4 Diagnostic Performance of LTWR and GGT in the Determination of Biliary Atresia**

| | Sensitivity | Specificity | PPV | NPV | Accuracy | OR |
|---|---|---|---|---|---|---|
| LTWR ≥5.2 | 60.97% | 95.12% | 92.59% | 70.90% | 78.04% | 30.469 |
| LTWR >4.75 | 68.29% | 92.68% | 90.32% | 74.51% | 80.48% | 27.282 |
| GGT >188 | 85.36% | 51.22% | 63.63% | 77.77% | 68.29% | 6.125 |
| GGT >390 | 70.73% | 78.04% | 76.31% | 72.72% | 74.39% | 8.593 |

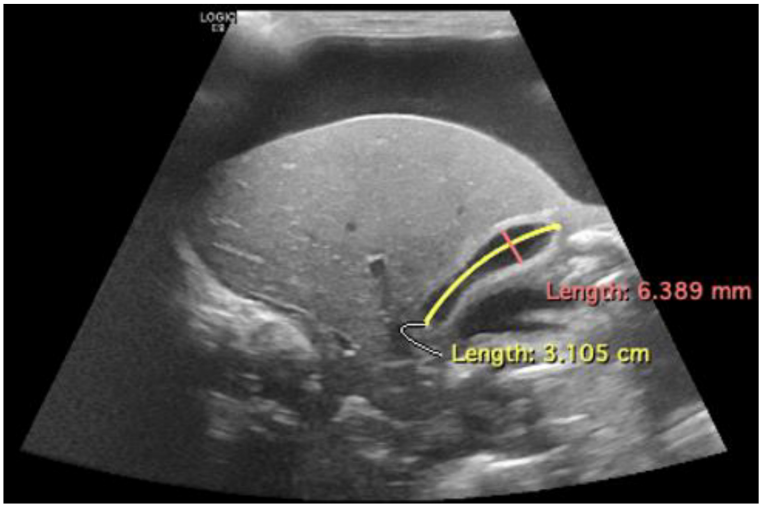


**Figure 2 Gallbladder LTWR Measurement Example**

the opposite is true. There is a theory that biliary atresia is an immune-mediated disease, as the frequency of human leukocyte antigen (HLA) B12 in atresia patients increases compared to cases. Epidemiologically, women are more likely to develop immune-mediated conditions compared to men.[12,13]

This study divides age into five age range groups, namely ≤30 days, 31–60 days, 61–90 days, 91–120 days, and ≥120 days. Most samples were in the age range ≥120 days, with 28 patients divided into 16 biliary atresia diagnoses and 12 non-biliary atresia diagnoses. This could be due to low awareness of biliary atresia and early diagnosis that cannot be done in primary health facilities. Therefore, referred patients to Dr. Soetomo Academic General Hospital are advanced patients. It is recommended that patients with suspicion of biliary atresia should be diagnosed at the age of <60 days because the age of recommendation for determining operative action in patients with biliary atresia is optimal in the age range of <60–90 days. This limitation is related to the success rate of post-treatment in biliary atresia cases. If there are findings that lead to cirrhosis, then operative action should ideally be carried out in the age range of 30–60 days; supporting examinations have a role in helping to establish a definitive diagnosis so that the principle of early diagnosis enforcement is prioritized.[3,5,14]

Abdominal ultrasound examinations routinely performed on neonatal cholestasis patients at Dr. Soetomo Academic General Hospital include triangular cord sign, subcapsular hepatic flow, and the ratio of the diameter of the hepatic artery to the portal vein, but in this study, the researchers added the variable length to width ratio of the gallbladder. The data processing results are presented alongside the results of the LTWR of the gallbladder, which was found to have a highly significant relationship ($p<0.001$) with biliary atresia. The above findings are similar to research conducted by Choochuen et al.,[15] showing the highest sensitivity in the length-to-width ratio of the gallbladder (71.7%) and the highest specificity in the triangular cord sign (95.9%). Other studies conducted by Zhang et al.[8] showed the superiority of the length-to-width ratio of the gallbladder. Although there is a slight difference in the results of data analysis, this can be caused by differences in the age distribution of the sample and the length-to-width ratio measurement technique. This study measured the outer to outer wall, as shown in Figure 2. In contrast, in Choochuen et al.,[15] measurements were made on the inner to inner wall.

LTWR of the gallbladder also offers a higher specificity value when compared to the triangular cord sign examination, so the researchers feel that at least LTWR of the gallbladder can complement the shortcomings of the triangular cord sign in the early detection of patients with suspected biliary atresia.

The literature agrees with a cut-off value for gallbladder LTWR of >5.2.6,[11] However, the researchers redetermined with a new cut-off result of >4.75. As shown in Table 4, there is an increase in sensitivity (68.29%) and negative predictive value (74.51%), which causes an increase in accuracy by 2%. Similar research by Zhang et al.[8] also redetermined the cut-off value with a sensitivity value (78.9%) and specificity (66.7%). The appearance of different cut-off values in this study can be caused by the process of ultrasound examination at Dr. Soetomo Academic General Hospital running simultaneously with other non-biliary atresia patients, so there is likely an additional fasting period of patients in this study with patients in other studies.[8,15]

This study analyzed the relationship between GGT in biliary atresia using a cut-off of >188 with sensitivity (85.36%), specificity (51.22%), PPV (63.63%), NPV (77.77%) and accuracy of 68.29% then recalculated the GGT cut-off with results >390, this recalculation caused an increase in specificity (78.04%) and positive predictive value (76.31%) which resulted in an increase in accuracy by 6% (74.39%).

The discrepancy in these findings may be attributed to variations in the demographic composition of the study sample relative to other studies. The current study's sample population was predominantly comprised of individuals within the age range of ≥121 days. In contrast, other studies conducted by Wang et al.[6] utilized a sample population within the age range of <30 days. In a survey conducted by Sun et al.,[9] 1,273 children were included in the analysis. The researchers utilized a cut-off value of >300 for biliary atresia patients. Despite discrepancies in the selected cut-off values, the diagnostic efficacy of GGT on the receiver operating characteristic (ROC) curve exhibited a comparable AUC value

range of 0.7-0.8, consistent with this study's findings.

The results of the data analysis indicate that there is no statistically significant correlation between the gallbladder's LTWR and GGT value. This finding suggests that an increase in LTWR does not necessarily correspond with a proportional increase or decrease in GGT.

GGT is agreed to be one of the potential diagnostic parameters for biliary atresia and suspicion of hepatotoxic conditions associated with liver injury and oxidative stress.[16] However, like other biomarkers, the value of GGT can be influenced by various circumstances, for example, the age range,[17,18] because in this study, the age of the sample was dominated in the age range >121 days, while in the studies conducted by Zhang et al.[8] the age of the sample was in the age range that had a significant relationship was the age group 31–90 days. As for the older age group in this study, it could be accompanied by the possibility of using ursodeoxycholic acid (UDCA), commonly applied to neonatal cholestasis patients.[17] Meanwhile, the researchers found that ultrasonographic findings were relatively more consistent when administering oral therapy. The diagnostic performance of LTWR and GGT value as single variables in assessing biliary atresia was statistically significant.[19,20] The combined use of biomarkers and ultrasound findings, especially LTWR and triangular cord signs, will undoubtedly increase the success rate in early diagnosis of biliary atresia.[21]

Additional research should be conducted using a prospective approach and a larger number of patients to obtain a more representative correlation. Further research could be carried out using age range group variables to ascertain the nature of the relationship between variables in greater detail. An additional avenue for research would be to employ a mixed methods approach, with agreed protocols and a multidisciplinary team, to obtain more representative samples and more homogeneous data.

## Conclusions

In this study, the gallbladder's LTWR has no significant relationship with the value of GGT, and the cut-off value has been recalculated to enhance the precision of each variable.

## Conflict of Interest

There is no ethical/legal conflict involved in the article. All authors have no relevant financial interest related to the material.

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## References

1. Schreiber RA, Barker CC, Roberts EA, Martin SR, Alvarez F, Smith L, et al. Biliary atresia: the Canadian experience. J Pediatr. 2007;151(6):659–65, 665.e1.
2. Adam A, Dixon AK, Gillard JH, Schaefer-Prokop CM, editors. Grainger & Allison's diagnostic radiology a textbook of medical imaging. 7th Edition. Warsaw, Poland: Elsevier Ltd.; 2021.
3. Setyoboedi B, Utomo MT, Prihaningtyas RA, Winahyu AK, Arief S. Tingkat pengetahuan atresia bilier pada bidan di puskesmas Kabupaten Sidoarjo. J Abdi Insani. 2022;9(4):1839–46.
4. arollo V, Gentile G, Di Piazza A, Marrone G, Milazzo M, Mamone G, et al. The role of ultrasound in the early diagnosis of biliary atresia [Internet]. Vienna, Austria: European Society of Radiology; 2018 [cited 2024 August 10]. Available from: https://dx.doi.org/10.1594/ecr2018/C-1472.
5. Napolitano M, Franchi-Abella S, Damasio MB, Augdal TA, Avni FE, Bruno C, et al. Practical approach to imaging diagnosis of biliary atresia, Part 1: prenatal ultrasound and magnetic resonance imaging, and postnatal ultrasound. Pediatr Radiol. 2021;51(2):314–31.
6. Wang G, Zhang N, Zhang X, Zhou W, Xie X, Zhou L. Ultrasound characteristics combined with gamma-glutamyl transpeptidase for diagnosis of biliary atresia in infants less than 30 days. Pediatr Surg Int. 2021;37(9):1175–82.
7. Sulistio PA, Violetta L, Rahniayu A. Diagnostic value of ultrasound parameter in neonatal

biliary atresia based on histopathological results (ultrasound study of triangular cord sign and gallbladder abnormality). Int J Res Publ. 2022;108(1):316–22.
8. Zhang K, Tang Y, Zheng Z, Tang C, Zhu D, Du Q, et al. Value of gallbladder length-to-width ratio for diagnosis of biliary atresia by correlation with age. Updates Surg. 2023; 75(4):915–20.
9. Sun Y, Dai S, Shen Z, Yang Y, Hong S, Dong R, et al. Gamma-glutamyl transpeptidase has different efficacy on biliary atresia diagnosis in different hospital patient groups: an application of machine learning approach. Pediatr Surg Int. 2022;38(8):1131–41.
10. Sira MM, Salem TAH, Sira AM. Biliary atresia: a challenging diagnosis. Glob J Gastroenterol Hepatol. 2013;1(1):24–35.
11. Zhou W, Zhou L. Ultrasound for the diagnosis of biliary atresia: from conventional ultrasound to artificial intelligence. MDPI. 2022;
12. Silveira TR, Salzano FM, Donaldson PT, Mieli-Vergani G, Howard ER, Mowat AP. Association between HLA and extrahepatic biliary atresia. J Pediatr Gastroenterol Nutr. 1993;16(2):114–7.
13. Black AJ, Lu DY, Yefet LS, Baird R. Sex differences in surgically correctable congenital anomalies: a systematic review. J Pediatr Surg. 2020;55(5):811–20.
14. Nio M, editor. Introduction to biliary atresia. Berlin: Springer Nature; 2021.
15. Choochuen P, Kritsaneepaiboon S, Charoonratana V, Sangkhathat S. Is "gallbladder length-to-width ratio" useful in diagnosing biliary atresia? J Pediatr Surg. 2019;54(9):1946–52.
16. Tang KS, Huang LT, Huang YH, Lai CY, Wu CH, Wang SM, et al. Gamma-glutamyl transferase in the diagnosis of biliary atresia. Acta Paediatr Taiwanica. 2007;48(4):196–200.
17. Kotb MA. Review of historical cohort: ursodeoxycholic acid in extrahepatic biliary atresia. J Pediatr Surg. 2008;43(7):1321–7.
18. Robles-Diaz M, Garcia-Cortes M, Medina-Caliz I, Gonzalez-Jimenez A, Gonzalez-Grande R, Navarro JM, et al. The value of serum aspartate aminotransferase and gamma-glutamyl transpetidase as biomarkers in hepatotoxicity. Liver Int. 2015;35(11):2474–82.
19. Simental-Mendía M, Sánchez-García A, Simental-Mendía LE. Effect of ursodeoxycholic acid on liver markers: a systematic review and meta-analysis of randomized placebo-controlled clinical trials. Br J Clin Pharmacol. 2020;86(8):1476–88.
20. Weng Z, Zhou W, Wu Q, Ma H, Fang Y, Dang T, et al. Gamma-glutamyl transferase combined with conventional ultrasound features in diagnosing biliary atresia: a two-center retrospective analysis. J Ultrasound Med. 2022;41(11):2805–17.
21. 21. Kong F, Dong R, Chen G, Sun S, Yang Y, Jiang J, et al. Progress in biomarkers related to biliary atresia. J Clin Transl Hepatol. 2024;12(3):305–15.

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