Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 24 July 2026
Revised 20 Aug 2026
Accepted 07 Sep 2026

Natural Resources for Human Health 2026, Vol. 6 (22s): 1719–1728

KEYWORDS:
Breastfeeding; lactational amenorrhea; menstrual pattern; postpartum; prolactin.

Serum Prolactin Levels and Menstrual Patterns among Breastfeeding Mothers: A Prospective Cohort Study at Three and Six Months Postpartum

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ABSTRACT: Prolactin mediates lactational amenorrhea by suppressing the hypothalamic–pituitary–ovarian axis, yet its quantitative relationship with postpartum menstrual patterns has rarely been evaluated in Indonesian women. This prospective cohort study was conducted in primary health centers in Makassar, Indonesia, from July to November 2025. Serum prolactin was measured by ELISA at 3 and 6 months postpartum, and menstrual status, volume, duration, and cycle length were obtained by structured interview. A prolactin cut-off was determined by ROC analysis, and associations were analyzed using chi-square or Fisher's exact tests and multivariable logistic regression.

Among 76 mothers, median prolactin decreased from 176.90 ng/mL at 3 months to 165.95 ng/mL at 6 months ($p < 0.001$). Prolactin at 3 months predicted amenorrhea with an AUC of 0.952 (95% CI 0.907–0.997); a cut-off of 196.5 ng/mL gave 80.6% sensitivity and 95.6% specificity. Prolactin ≥196.5 ng/mL was associated with amenorrhea at 3 months (25/27 vs. 6/49) and 6 months (19/20 vs. 7/56; both $p < 0.001$), but not with menstrual volume or duration. Frequent (≥8 times/day) and exclusive breastfeeding were associated with higher prolactin and amenorrhea ($p < 0.001$), but only prolactin remained independently associated with amenorrhea (OR 1.116 per 1 ng/mL; 95% CI 1.023–1.217; $p = 0.013$). Serum prolactin is the most proximal determinant of postpartum amenorrhea, supporting counseling on breastfeeding intensity when the lactational amenorrhea method is used.

1. INTRODUCTION

Lactation is a complex physiological period characterized by profound hormonal adaptation in the postpartum mother. Prolactin, a peptide hormone secreted by the anterior pituitary gland, plays a central role in the initiation and maintenance of milk production. The rise in prolactin induced by infant suckling inhibits the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby suppressing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and preventing ovulation. This state of physiological hyperprolactinemia underlies lactational amenorrhea [1]. Prolactin therefore acts as a key regulator of the balance between lactation and reproductive function, with important implications for maternal reproductive health and natural fertility regulation [2].

At the neuroendocrine level, prolactin suppresses the activity of kisspeptin neurons in the arcuate nucleus, which drive GnRH pulsatility [1,3,4]. As breastfeeding frequency declines, prolactin concentrations fall progressively, allowing the resumption of ovulation and menstruation [1]. The duration of lactational amenorrhea and the timing of menstrual return vary widely and are

influenced by breastfeeding frequency, nutritional status, psychological stress, and environmental conditions [5]. Beyond lactation, prolactin also exerts systemic metabolic and psychoneuroendocrine effects, including modulation of the stress response and maternal behavior, and psychological stress has been shown to lower prolactin concentrations in both blood and milk [5,6].

Recent studies have reported mean serum prolactin levels exceeding 120 ng/mL among exclusively breastfeeding mothers, markedly higher than in mothers who breastfeed partially or not at all, with a decline typically observed after six months as complementary feeding is introduced [7]. In a large Japanese cohort, delayed resumption of menstruation beyond 72 weeks postpartum occurred in approximately 8% of breastfeeding women, with persistent breastfeeding identified as a dominant associated factor [8]. These findings indicate that the relationship between prolactin and menstrual patterns is dynamic and shaped by both physiological and behavioral factors.

In Indonesia, this relationship is of particular relevance given the persistently high birth rate and ongoing efforts to promote natural contraceptive methods such as the lactational amenorrhea method (LAM). LAM is approximately 98% effective in preventing pregnancy when three criteria are met: the mother remains amenorrheic, the infant is fully or nearly fully breastfed, and the infant is younger than six months [9]. However, limited understanding of the physiology of lactational amenorrhea often leads to inappropriate use of LAM, increasing the risk of unintended pregnancy [10,11]. Although the role of breastfeeding in delaying the return of menses is well established, few studies have quantified the association between serum prolactin concentration and specific menstrual characteristics at defined postpartum time points in Indonesian women. This study therefore aimed to evaluate the association between serum prolactin levels and menstrual patterns among breastfeeding mothers at 3 and 6 months postpartum, and to examine the influence of breastfeeding status and frequency on prolactin levels and menstrual patterns.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This was a prospective observational cohort study in which serum prolactin and menstrual patterns were assessed at 3 and 6 months postpartum without any intervention. The study was conducted at several primary health centers (Puskesmas) in Makassar, Indonesia, that serve active breastfeeding populations, with serum prolactin measured at the referral laboratory of a teaching hospital affiliated with the Faculty of Medicine, Hasanuddin University. Data were collected from July to November 2025.

2.2 Participants

The target population comprised all breastfeeding postpartum mothers with infants aged 3 months in the working area of primary health centers in Makassar. Participants were recruited by consecutive non-probability sampling among mothers attending the selected health centers during the study period. Inclusion criteria were: (1) maternal age 18–45 years; (2) active breastfeeding, either exclusive or non-exclusive; (3) residence and attendance at a participating primary health center or affiliated hospital; (4) good general health and willingness to provide written informed consent; and (5) no use of hormonal contraception from delivery until 6 months postpartum.

Exclusion criteria were: (1) a history of endocrine disorders, including hypothyroidism, pathological hyperprolactinemia, Sheehan syndrome, or polycystic ovary syndrome; (2) use of medications affecting prolactin levels, such as domperidone, metoclopramide, antidepressants, or antipsychotics; (3) psychotic disorders or severe postpartum stress (Edinburgh Postnatal Depression Scale score >13); (4) autoimmune disease or chronic kidney failure; and (5) inability to breastfeed directly, for example because of severe mastitis or congenital anomalies of the infant. Participants who withdrew consent, missed the 6-month evaluation, had unanalyzable blood samples, or had incomplete data on the main variables were considered dropouts.

2.3 Sample Size

The sample size was calculated using the formula for comparing two proportions in a cohort design, with a two-sided α of 0.05, a power of 80%, and expected proportions of amenorrhea of 0.70 and 0.35 in the high- and low-prolactin groups, respectively. This yielded a minimum of 28 participants per group, which was increased to 33 per group (66 in total) to anticipate dropouts and incomplete data [12–14].

Natural Resources for Human Health
eISSN: 2583-1194

2.4 Variables and Measurements

The independent variable was serum prolactin concentration (ng/mL). Venous blood samples were drawn in the morning at 3 and 6 months postpartum, and serum prolactin was measured using an enzyme-linked immunosorbent assay (ELISA). The dependent variable was the menstrual pattern, which comprised menstrual status (amenorrhea or resumed menstruation), menstrual volume (<30 mL, 30–80 mL, or >80 mL, estimated from the number of sanitary pads used), menstrual duration (<3 days, 3–7 days, or >7 days), and cycle length (<21 days, 21–35 days, or >35 days). Menstrual data were obtained through structured interviews. Covariates included breastfeeding status, defined as exclusive (direct breastfeeding without any additional fluids or foods) or non-exclusive (receipt of formula, other fluids, or solid foods), and breastfeeding frequency, defined as the number of direct breastfeeds in 24 hours (<8 times per day or ≥8 times per day on demand). Maternal age, parity, mode of delivery, and body mass index were also recorded.

2.5 Statistical Analysis

Data were entered and analyzed using IBM SPSS Statistics version 31 (IBM Corp., Armonk, NY, USA). The normality of continuous data was assessed using the Kolmogorov–Smirnov test with Lilliefors correction because the sample size exceeded 50; non-normally distributed data are presented as medians (minimum–maximum), and prolactin levels at 3 and 6 months were compared using the Wilcoxon signed-rank test. A prolactin cut-off value for predicting amenorrhea was determined by receiver operating characteristic (ROC) curve analysis of prolactin at 3 months postpartum, with the area under the curve (AUC) reported with its 95% confidence interval; the same cut-off was applied at 6 months, and prolactin was subsequently analyzed as a categorical variable. Associations between categorical variables were assessed using the chi-square test, or Fisher's exact test when any expected cell count was below 5. Variables associated with menstrual status in bivariate analysis were entered into a multivariable logistic regression model to identify independent factors, and results are presented as odds ratios (OR) with 95% confidence intervals (CI). A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Participant Characteristics

A total of 76 breastfeeding mothers were included in the analysis. Their characteristics are summarized in Table 1. Most participants were aged 20–35 years (72.4%), were primiparous (56.6%), and had delivered vaginally (72.4%). Most mothers were breastfeeding non-exclusively (65.8%) and fewer than eight times per day (57.9%) at 6 months postpartum. Three-quarters of participants had a normal body mass index. Among the 50 mothers whose menstruation had resumed by 6 months, most had a cycle length of 21–28 days (72.0%).

Table 1. Characteristics of study participants (n = 76)

Characteristic	n	%
Maternal age (years)		
<20 (all aged 19)	10	13.2
20–35	55	72.4
>35	11	14.5
Parity		
Primiparous	43	56.6
Multiparous	33	43.4
Breastfeeding status		
Exclusive	26	34.2
Non-exclusive	50	65.8
Breastfeeding frequency at 6 months (times/day)		
<8	44	57.9
≥8 (on demand)	32	42.1
Menstrual cycle length at 6 months (n = 50)[a]		
<21 days	1	2

21–28 days	36	72
>28 days	13	26
Mode of delivery		
Vaginal delivery	55	72.4
Cesarean section	21	27.6
Body mass index		
Underweight	2	2.6
Normal	57	75
Overweight	12	15.8
Obese	5	6.6
Total	76	100

[a] Percentages calculated among the 50 mothers whose menstruation had resumed at 6 months postpartum.

3.2 Serum Prolactin Levels

Serum prolactin levels at both time points are presented in Table 2. Prolactin levels were not normally distributed at either time point (Kolmogorov–Smirnov $p < 0.001$ at 3 months and $p = 0.004$ at 6 months). The median prolactin level was 176.90 ng/mL (range 109.0–299.0 ng/mL; mean 193.17 ± 55.09 ng/mL) at 3 months postpartum and decreased significantly to 165.95 ng/mL (range 100.0–299.0 ng/mL; mean 174.73 ± 46.94 ng/mL) at 6 months postpartum (Wilcoxon signed-rank test, $Z = -4.656$; $p < 0.001$). In ROC analysis, prolactin at 3 months discriminated amenorrheic from menstruating mothers with an AUC of 0.952 (95% CI 0.907–0.997; $p < 0.001$). A cut-off of 196.5 ng/mL yielded a sensitivity of 80.6% and a specificity of 95.6% and was used for subsequent analyses.

Table 2. Serum prolactin levels at 3 and 6 months postpartum

Time point	n	Median (ng/mL)	Minimum	Maximum	Mean ± SD (ng/mL)	p-value
3 months postpartum	76	176.90	109.0	299.0	193.17 ± 55.09	<0.001[a]
6 months postpartum	76	165.95	100.0	299.0	174.73 ± 46.94	

SD, standard deviation. Prolactin was not normally distributed (Kolmogorov–Smirnov $p < 0.001$ and $p = 0.004$). [a] Wilcoxon signed-rank test, 3 vs. 6 months ($Z = -4.656$).

3.3 Association between Prolactin Level and Menstrual Pattern

At 3 months postpartum, 25 of 27 mothers (92.6%) with prolactin ≥196.5 ng/mL were amenorrheic, compared with 6 of 49 mothers (12.2%) with prolactin <196.5 ng/mL ($p < 0.001$) (Table 3). Among the 45 mothers whose menstruation had resumed, prolactin level at 3 months was not significantly associated with menstrual volume ($p = 0.290$) or menstrual duration ($p = 0.088$); only two of these mothers had prolactin ≥196.5 ng/mL.

Table 3. Association between serum prolactin at 3 months postpartum and menstrual pattern

Variable	Category	≥196.5 ng/mL	<196.5 ng/mL	Total	p-value
Menstrual status	Menstruating	2	43	45	<0.001
	Amenorrhea	25	6	31	
Menstrual volume[b]	<30 mL	1	6	7	0.290
	30–80 mL	1	37	38	
Menstrual duration[b]	<3 days	1	1	2	0.088
	3–5 days	1	42	43	

Pearson chi-square for menstrual status; Fisher's exact test for volume and duration. [b] Among the 45 mothers whose menstruation had resumed.

At 6 months postpartum, 19 of 20 mothers (95.0%) with prolactin ≥196.5 ng/mL remained amenorrheic, compared with 7 of 56 mothers (12.5%) with lower prolactin levels ($p < 0.001$) (Table 4). Among the 50 mothers with resumed menstruation, only one had a prolactin level ≥196.5 ng/mL; therefore, the associations between prolactin and menstrual volume, duration, and cycle length could not be tested because of insufficient cell counts.

Table 4. Association between serum prolactin at 6 months postpartum and menstrual pattern

Variable	Category	≥196.5 ng/mL	<196.5 ng/mL	Total	p-value
Menstrual status	Menstruating	1	49	50	<0.001
	Amenorrhea	19	7	26	
Menstrual volume[b]	<30 mL	1	7	8	N/A[c]
	30–80 mL	0	41	41	
	>80 mL	0	1	1	
Menstrual duration[b]	<3 days	0	8	8	N/A[c]
	3–5 days	1	41	42	
Cycle length[b]	<21 days	0	1	1	N/A[c]
	21–28 days	1	35	36	
	>28 days	0	13	13	

Chi-square or Fisher's exact test. [b] Among mothers whose menstruation had resumed. [c] Not analyzed because of insufficient cell counts.

3.4 Breastfeeding Frequency, Breastfeeding Status, Prolactin Level, and Menstrual Pattern

Breastfeeding frequency was significantly associated with prolactin level and menstrual status (Table 5). Among mothers who breastfed ≥8 times per day, 19 of 32 (59.4%) had prolactin ≥196.5 ng/mL and 24 of 32 (75.0%) were amenorrheic, compared with 1 of 44 (2.3%) and 2 of 44 (4.5%), respectively, among those who breastfed fewer than eight times per day (both $p < 0.001$). Breastfeeding frequency was not associated with menstrual volume ($p = 0.598$) or duration ($p = 1.000$).

Table 5. Association between breastfeeding frequency and prolactin level and menstrual pattern at 6 months postpartum

Variable	Category	<8 times/day	≥8 times/day	Total	p-value
Prolactin[d]	≥196.5 ng/mL	1	19	20	<0.001
	<196.5 ng/mL	43	13	56	
Menstrual status	Menstruating	42	8	50	<0.001
	Amenorrhea	2	24	26	
Menstrual volume[b]	<30 mL	6	2	8	0.598
	≥30 mL	36	6	42	
Menstrual duration[b]	<3 days	7	1	8	1.000
	3–5 days	35	7	42	

Chi-square or Fisher's exact test. [b] Among the 50 mothers whose menstruation had resumed; volume categorized as <30 vs. ≥30 mL. [d] Serum prolactin at 6 months postpartum.

Similarly, exclusive breastfeeding was associated with higher prolactin levels and a higher proportion of amenorrhea (both $p < 0.001$) (Table 6). Of the 26 exclusively breastfeeding mothers, 15 had prolactin ≥196.5 ng/mL and 19 were amenorrheic, whereas among the 50 non-exclusively breastfeeding mothers, 5 had high prolactin levels and 7 were amenorrheic. Breastfeeding status was not associated with menstrual volume or duration (both $p = 1.000$).

Table 6. Association between breastfeeding status and prolactin level and menstrual pattern at 6 months postpartum

Variable	Category	Exclusive	Non-exclusive	Total	p-value
Prolactin[d]	≥196.5 ng/mL	15	5	20	<0.001
	<196.5 ng/mL	11	45	56	
Menstrual status	Menstruating	7	43	50	<0.001
	Amenorrhea	19	7	26	
Menstrual volume[b]	<30 mL	1	7	8	1.000
	≥30 mL	6	36	42	
Menstrual duration[b]	<3 days	1	7	8	1.000
	3–5 days	6	36	42	

Chi-square or Fisher's exact test. [b] Among the 50 mothers whose menstruation had resumed; volume categorized as <30 vs. ≥30 mL. [d] Serum prolactin at 6 months postpartum.

3.5 Multivariable Analysis

In multivariable logistic regression including serum prolactin, breastfeeding frequency, and breastfeeding status, serum prolactin was the only variable independently associated with amenorrhea ($\beta = 0.110$; OR 1.116; 95% CI 1.023–1.217; $p = 0.013$) (Table 7). Each 1 ng/mL increase in prolactin was associated with an 11.6% increase in the odds of amenorrhea after adjustment for breastfeeding frequency and status. Neither breastfeeding frequency (OR 0.222; 95% CI 0.011–4.600; $p = 0.331$) nor breastfeeding status (OR 0.373; 95% CI 0.014–10.265; $p = 0.560$) remained significant after adjustment.

Table 7. Multivariable logistic regression of factors associated with amenorrhea

Variable	β	SE	p-value	OR	95% CI
Serum prolactin (per 1 ng/mL)	0.110	0.044	0.013	1.116	1.023–1.217
Breastfeeding <8 times/day (ref: ≥8)	−1.503	1.546	0.331	0.222	0.011–4.600
Non-exclusive breastfeeding (ref: exclusive)	−0.987	1.690	0.560	0.373	0.014–10.265

Outcome: amenorrhea at 6 months postpartum. β, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; ref, reference category. Nagelkerke $R^2 = 0.846$.

3.6 Discussion

This prospective cohort study demonstrated that serum prolactin was strongly associated with lactational amenorrhea at both 3 and 6 months postpartum, and that it was the only factor that remained independently associated with amenorrhea after adjustment for breastfeeding frequency and status. Prolactin showed excellent discrimination for amenorrhea (AUC 0.952), and a threshold of 196.5 ng/mL identified amenorrheic mothers with high specificity. Breastfeeding frequency and exclusivity were associated with both prolactin levels and amenorrhea in bivariate analysis, but lost significance in the multivariable model.

The strong association between high prolactin and amenorrhea is consistent with the established neuroendocrine basis of lactational infertility. Prolactin suppresses GnRH release by inhibiting kisspeptin neurons in the arcuate nucleus of the hypothalamus, leading to reduced LH and FSH secretion and anovulation [3,4]. Experimental evidence has shown that prolactin-mediated suppression of arcuate kisspeptin neuronal activity is necessary for lactational infertility [3]. In the present study, nearly all mothers with prolactin ≥196.5 ng/mL remained amenorrheic, indicating that prolactin is a strong determinant of the timing of menstrual resumption. These findings agree with previous studies showing that breastfeeding intensity and hormonal factors play significant roles in delaying the return of postpartum menstruation [15,16].

Among mothers whose menstruation had resumed at 3 months, prolactin level was not significantly associated with menstrual volume or duration. This is likely explained by the very small number of menstruating mothers with high prolactin (n = 2), which limited statistical power, rather than by an absence of biological effect. Early postpartum cycles are frequently anovulatory or accompanied by an inadequate luteal phase, resulting in suboptimal progesterone production and shorter, lighter bleeding [17,18]. The return of menses at 3 months therefore does not necessarily reflect full recovery of reproductive function. Persistent prolactin elevation and incomplete restoration of stable GnRH pulsatility may explain the irregular and prolonged cycle intervals observed during this period [1,8].

The decline in prolactin from 3 to 6 months postpartum reflects physiological endocrine adaptation as infants grow and breastfeeding patterns change. Prolactin rises sharply in late pregnancy, remains elevated during lactation, and gradually decreases as suckling stimulation diminishes [19]. By 6 months, most mothers with lower prolactin had resumed menstruation with a normal volume of 30–80 mL, although a small proportion still reported lighter bleeding. This suggests that although a fall in prolactin permits menstrual resumption, full recovery of endometrial function and hormonal stability may not yet have been achieved in all women, as the endometrium adapts to fluctuating estrogen and progesterone stimulation [18,19].

Breastfeeding ≥8 times per day and exclusive breastfeeding were both associated with higher prolactin levels and a higher prevalence of amenorrhea at 6 months postpartum. More frequent nipple stimulation produces greater episodic prolactin surges, sustaining suppression of the hypothalamic–pituitary–ovarian axis [2]. These results are consistent with Afolabi and Afolabi, who reported a longer interval to menstrual return among exclusively breastfeeding women [20], and with Wati et al., who found significantly higher serum prolactin among Indonesian mothers who breastfed more frequently [7]. Samejima et al. likewise reported that delayed menstrual resumption was associated with exclusive and high-frequency breastfeeding, particularly during the first six months [8]. Together, these observations are in line with the LAM concept, in which frequent, exclusive breastfeeding maintains prolactin at levels sufficient to suppress ovulation for up to six months after delivery [9,20].

The loss of significance of breastfeeding frequency and status after adjustment suggests that the effect of breastfeeding on amenorrhea is not direct but is mediated through increased prolactin secretion. Infant suckling triggers a neuroendocrine reflex that elevates prolactin, which in turn suppresses gonadotropin release and ovulation [1,3]. Serum prolactin is therefore the more

proximal biological determinant of amenorrhea, whereas breastfeeding behavior acts upstream. The very wide confidence intervals for breastfeeding frequency and status in the regression model also reflect the strong collinearity between these variables and prolactin, as well as the small number of discordant observations, and should be interpreted with caution.

Some previous studies have reported heterogeneous findings. An analysis of Ethiopian Demographic and Health Survey data found that, in addition to breastfeeding, maternal nutritional status, age, and postpartum contraceptive use influenced the timing of menstrual return [16]. Eleje et al. suggested that individual variation in hypothalamic–pituitary–ovarian sensitivity to prolactin may allow some women with high prolactin to resume menstruation earlier than expected [15]. Differences in population characteristics, including nutritional status, stress levels, and access to health care, as well as differences in study design and duration of follow-up, may account for these discrepancies. Nevertheless, the consistent finding across studies is that prolactin and breastfeeding intensity play central roles in lactational amenorrhea.

From a clinical perspective, these findings reinforce the importance of counseling mothers on breastfeeding frequency and exclusivity when LAM is used for birth spacing, and of recommending a transition to another contraceptive method when breastfeeding intensity declines or menstruation returns [9,11]. Serum prolactin measurement is not required for routine LAM counseling, but the observed threshold provides biological support for the behavioral criteria of LAM.

This study has several strengths. Menstrual patterns were assessed comprehensively, including amenorrhea, volume, duration, and cycle length, rather than menstrual resumption alone. Prolactin was measured objectively at two postpartum time points, allowing evaluation of the consistency of the association over time, and multivariable analysis was used to identify independent factors. The study was conducted in primary care settings, enhancing its relevance to routine maternal health services.

Several limitations should also be acknowledged. First, prolactin was not measured in the early puerperium, during pregnancy, or before conception, precluding assessment of baseline levels and more detailed longitudinal dynamics. Second, no control group of non-breastfeeding postpartum mothers was included, so the effect of prolactin could not be fully separated from that of breastfeeding itself. Third, several cross-tabulations contained cells with very small or zero counts, which limited statistical testing of menstrual characteristics and widened the confidence intervals in the regression model. Fourth, menstrual volume was estimated from self-reported pad use and is subject to recall bias. Finally, other factors that may influence menstrual resumption, such as nutritional status, psychological stress, and other reproductive hormones, were not evaluated.

4. CONCLUSION

Serum prolactin was significantly associated with postpartum amenorrhea among breastfeeding mothers at 3 and 6 months postpartum, and it was the only factor independently associated with amenorrhea after adjustment for breastfeeding frequency and status. Frequent and exclusive breastfeeding were associated with higher prolactin levels and amenorrhea, likely acting through prolactin secretion. These findings support the physiological basis of the lactational amenorrhea method and highlight the importance of counseling on breastfeeding intensity for postpartum birth spacing. Future studies should include non-breastfeeding control groups, serial prolactin measurements from the early postpartum period, and assessment of other reproductive hormones.

ACKNOWLEDGEMENTS

The authors thank all participating mothers and the staff of the primary health centers in Makassar for their support during data collection.

AUTHOR CONTRIBUTIONS

Akbar Yunus: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing—Original Draft

Nusratuddin Abdullah: Supervision, Methodology, Writing—Review & Editing

Ajardiana: Supervision, Validation, Writing—Review & Editing

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Approval Number: 711/UN4.6.45.31/PP36/2025). All participants provided written informed consent before enrollment.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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