RESEARCH ARTICLE

Presepsin Levels and Neutrophil-to-Lymphocyte Ratio are Positively Correlated with Procalcitonin Levels in Early Onset Neonatal Sepsis

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Abstract

BACKGROUND: Early-onset neonatal sepsis (EONS) is an acute infection and sepsis occurring within the first 24 hours of new-born life. An increase in procalcitonin levels, presepsin levels, and neutrophil-to-lymphocyte ratio (NLR) in EONS subjects have been reported. However, whether presepsin levels and NLR affect the procalcitonin levels in EONS patients who have received antibiotic therapy has not been certainly known. This study was conducted to determine the correlation between presepsin levels and NLR with procalcitonin levels in EONS subjects.

METHODS: A cross-sectional study involving 52 EONS subjects were conducted, and blood samples from subjects were collected. Presepsin levels were examined by enzyme-linked immunosorbent assay (ELISA) method, NLR was calculated from the absolute number of neutrophils divided by the absolute number of lymphocytes, and procalcitonin levels were examined by chemiluminescent microparticle immunoassay (CMIA) method.

RESULTS: Median of procalcitonin levels and presepsin levels were 0.435 (0.12-9.11) ng/mL and 108.33 (71.43-1287.76) ng/L, respectively. While NLR value was 1.68 (0.2-7.52). There was significant difference between procalcitonin and presepsin levels (p=0.000), and so does between procalcitonin levels and NLR (p=0.001). Based on the multivariate analysis, presepsin levels also affected the procalcitonin levels (p=0.000; 95% CI: 0.001-0.004).

CONCLUSION: The results of the study showed that there was significant correlation between presepsin levels and NLR with procalcitonin levels in EONS patients, suggesting that presepsin levels, NLR, and procalcitonin levels are potential candidates for EONS biomarkers.

KEYWORDS: EONS, NLR, presepsin, procalcitonin

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Introduction

The neonatal period is the most vulnerable period for neonatal survival with a global mortality rate of 17 deaths per 1000 live births in 2019. About 15% of neonatal death was caused by sepsis.(1-3) Neonatal sepsis is a clinical syndrome that arises as a result of the systemic inflammatory response syndrome (SIRS) that occurs due to bacterial, viral, fungal, or parasitic infections that occur in the neonatal period.(4-6) Neonatal sepsis is divided into two groups, early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis

(LONS) based on the time of onset of sepsis. Early onset neonatal sepsis occurs within 72 hours of birth and LONS occurs after 72 hours of birth to 28 days.(7,8)

Positive blood culture is the gold standard for diagnosing neonatal sepsis, but it has several obstacles, such as it takes time to arrive 72 hours to obtain test results, possible false negative results with previous antepartum or empiric antibiotic therapy, insufficient blood sample size, and inability to produce microorganisms can cause delays and errors in diagnosis.(8,9)

Procalcitonin is an acute phase marker of systemic reactions. Exogenous stimulation by molecules such as cytokines and lipopolysaccharide (LPS), as well as chemokines for blood monocytes, causes procalcitonin to be secreted by various tissues.(10,11) Developed strategies to improve diagnosis and assess rates of the severity of infection is still needed to guide physician decisions. Markers with better specificity for systemic responses to microorganisms can be found among the molecules involved in the immune response.(12)

A new diagnostic marker for sepsis is presepsin or soluble subtype 14 differentiation cluster (sCD14-ST). Presepsin is not only a marker for the diagnosis of sepsis but also a prognostic marker for sepsis. High levels of presepsin indicate a higher mortality rate in patients with neonatal sepsis.(13,14) The release of presepsin and increased production of procalcitonin by monocytes or macrophages occur after the body's natural response to bacterial infection. A cluster of differentiation 14 (CD14) activates toll-like receptor 4 (TLR4) which is a specific proinflammatory signalling cascade to initiate reactions inflammation against microorganisms. Presepsin is released after the occurrence of CD14-LPS binding on monocytes or macrophages, whereas procalcitonin is produced after monocytes or macrophages release cytokines proinflammatory, such as interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α or directly stimulated by bacterial LPS.(15,16)

The white blood cell population in immunocompetent patients (monocytes, lymphocytes, and neutrophils) plays an important role in the systemic inflammatory response to heavy infection. The hyperdynamic phase in early infection is characterized by a proinflammatory status mediated by neutrophils, macrophages, and monocytes that follow the release of inflammatory cytokines. This systemic inflammatory response is associated with suppression of enhanced neutrophil apoptosis neutrophil-mediated killing of pathogens as part of the innate immune response but can also cause tissue damage. When concomitantly, lymphocyte apoptosis in the thymus gland and spleen increases. This can lead to suppression of the immune system, multiple organ dysfunction, and death.(17)

Procalcitonin is correlated with infection. Presepsin is released as the body's natural response against bacterial infection, whereas NLR is an inflammatory biomarker that can be used as an indicator of systemic inflammation. (11,16,17) There has not been much research on the relationship between presepsin and NLR with procalcitonin in subjects with neonatal sepsis. Based on the guidelines for neonatal sepsis at Sanglah Hospital, procalcitonin is checked if the patient suspected sepsis is ≥3 days old or after 72 hours of antibiotic administration there is no clinical improvement.(4) The neutrophil-to-lymphocyte ratio (NLR) can be counted easily and can be obtained quickly from a complete blood count as part of a routine laboratory.(17) Examination of NLR, presepsin and procalcitonin in another study was carried out after the onset of sepsis and before giving antibiotics. Therefore, this study was conducted to determine whether there is a remaining relationship between presepsin levels and NLR with procalcitonin levels in EONS subjects who have received antibiotic therapy, hence can be used in monitoring therapy.

Methods

Study Design and Subject Recruitment

An observational analytic study with a cross-sectional study design was conducted between February to June 2022, involving 52 EONS patients treated at the Cempaka 1 Neonatal Intensive Care Unit (NICU) and Neonatal Inpatient Installation, Sanglah Hospital, Denpasar. Subjects with incomplete medical record data or neonates with congenital heart disease were excluded from the study. The sample size was determined based on the sample size for the correlation coefficient.(18) The minimum total samples required were 47. To avoid a loss to follow-up, the number of samples was increased by 10% to 52 samples. This study protocol has been approved by The Research Ethics Committee of the Faculty of Medicine, Universitas Udayana (No. 231/UN14.2.2.VII.14/LT/2022).

Neonatal Sepsis Condition

The diagnosis of neonatal sepsis was the presence of infection risk factors (at least one risk of major infection or two risks of minor infection), clinical and physical examination, and laboratory tests (obtained at least positive on two examinations with or without a positive blood culture result). The major risk factors for infection were

the rupture of membranes >24 hours, the mother had fever during intrapartum temperature >38º C, chorioamnionitis, fetal heart rate persisting >160 beats/minute, and amniotic fluid smells. Meanwhile, the minor risk factors for infection were rupture of membranes >12 hours, mother had fever during intrapartum temperature >37.5º C, low Apgar score (1st minute < 5; 5th minute < 7), very low birth weight <1500 grams, gestational age <37 weeks, multiple pregnancies, untreated discharge, and untreated urinary tract infection (UTI) or suspected UTI.(4)

Laboratory Analysis

The laboratory tests for neonatal sepsis were including the leucocyte count <5000 or >35,000/µL, neutropenia <1500/µL or neutrophilia, platelets <150.000/µL, procalcitonin ≥0.05 ng/mL, IT ratio >0.2, peripheral blood smear (positive vacuolization and/or toxic granules), and blood culture.(4)

Data on the results of leucocyte count, neutrophil count, platelet count, IT ratio, peripheral blood smear, and blood culture were taken from medical record data when the signs and symptoms of sepsis begin dan before giving antibiotics. The hematology analyzer used was Sysmex XN-3000 (Sysmex, Kobe, Japan), and DI-60 (Sysmex) and the peripheral blood smear was examined under a microscope.

Examination of Procalcitonin

Procalcitonin was checked after 72 hours of antibiotic administration if there was no clinical improvement. One to two mL venous blood sample was inserted into a plain tube. After the blood clots, samples were centrifuged at 3000 RPM for 20 minutes. The serum volume for the test was 150 µL. Examination of procalcitonin was carried out on the Alinity device (Abbott, Chicago, IL, USA) using a two-step immunoassay with the chemiluminescent microparticle immunoassay (CMIA) method. Sample and anti-procalcitonin coated paramagnetic microparticles were combined and incubated. The procalcitonin present in the sample binds to the anti-procalcitonin-coated microparticles, and washed. Anti-procalcitonin acridinium-labelled conjugate was added to create a reaction mixture and incubated. Following a wash cycle, pre-trigger and trigger solutions were added. The resulting chemiluminescent reaction was measured as relative light units (RLUs). There was a direct relationship between the amount of procalcitonin in the sample and the RLUs detected by the system optics. The result was the procalcitonin level present in the serum sample with the measuring interval 0.02 to 100 ng/mL. The reagent and control used were the Alinity i-BRAHMS PCT Reagent Kit and Alinity i-BRAHMS PCT Controls (Abbott).(19,20)

Examination of Presepsin

Blood collection for presepsin examination was carried out in conjunction with procalcitonin examination to minimize puncture. Sixty until one hundred µL serum was inserted into a sample cup and stored at -80°C for presepsin examination. Avoid freeze-thaw on samples. Samples were prepared at room temperature when the inspection was about to be carried out. Presepsin serum was examined using the sandwich enzyme-linked immunosorbent assay (ELISA) method with human presepsin ELISA kit (Bioassay Technology Laboratory, Shanghai, China). The plate had been pre-coated with a Human Presepsin antibody (Bioassay Technology Laboratory). Presepsin present in the sample was added and binds to antibodies coated on the wells. And then biotinylated human presepsin antibody was added and binds to presepsin in the sample. Then Streptavidin-HRP was added and binds to the biotinylated presepsin antibody. After incubation unbound Streptavidin-HRP had been washed away during a washing step. A substrate solution was then added, and color developed in proportion to the amount of human presepsin. The reaction was terminated by the addition of an acidic stop solution and absorbance was measured at 450 nm.(21)

Calculation of NLR

The NLR was defined by the absolute number of neutrophils divided by the absolute number of lymphocytes.(22) The NLR data was taken from the medical record of the complete blood count examination on the same day as the procalcitonin examination.

Statistical Analysis

The data analysis was performed using SPSS 25.0 software (IBM Corporation, Armonk, NY, USA). The results of statistical analysis were presented in a single distribution table. Kolmogorov Smirnov test was performed for the normality test. The data distribution was normally distributed if the $p>0.05$. Multiple linear regression test was performed to assess the relationship between presepsin and procalcitonin levels after controlling for confounding variables analytically. The inference process or conclusion was based on 95% confidence intervals and p-values (significant if $p<0.05$).

Results

Study subjects' characteristics were obtained from the medical record data, and shown in Table 1. Meanwhile, the characteristics of study subjects based on risk factors for infection in EONS subjects could be seen in Table 2. The number of subjects born prematurely was quite high (73.1%).

Clinical signs that were observed from subjects were hyperthermia (5.8%), hypothermia (3.8%), lethargy (26.9%), changes in tone (34.6%), jaundice (11.5%), cyanosis (17.3%), poor peripheral perfusion (1.9%), flatulence (9.6%), vomiting (5.8%), respiratory distress (90.4%), hyperglycemia (7.7%), and hypoglycemia (5.8%) (Table 3).

Characteristics of study subjects based on the laboratory examinations were presented in Table 4. Procalcitonin levels and presepsin levels were 0.435 (0.12-9.11) ng/mL and 108.33 (71.43-1287.76) ng/L, respectively. While NLR value was 1.68 (0.2-7.52).

Since the data were not normally distributed, Spearman correlation analysis was used for the correlation test of NLR, presepsin levels, and procalcitonin levels. There was significant difference between procalcitonin and presepsin levels (p=0.000), and so does between procalcitonin levels and NLR (p=0.001) (Table 5).

In addition to the correlation coefficient, a scatter plot was also created to visualize the direction and strength of the correlation. Figure 1 showed a scatter plot of the relationship between presepsin and procalcitonin levels. The direction of the line on the scatter plot indicates a positive correlation with linear r^2=0.199, which mean that the variability of the procalcitonin levels of 19.9% was influenced by presepsin levels and the rest was influenced by other factors. Meanwhile, Figure 2 showed a scatter plot of the relationship between NLR and procalcitonin levels. There was a positive correlation with linear r^2=0.084 meaning that the variability of the procalcitonin levels of 8.4% was influenced by NLR value and the rest was influenced by other factors.

Table 6 showed the results of multivariate analysis with multiple linear regression tests. Presepsin levels affected procalcitonin levels after controlling for confounding

Table 1. Characteristics of study subjects (n=52).

Variable	Value
Age (days), n (%)	
One	30 (57.7)
Two	7 (13.5)
Three	15 (28.8)
Gender, n (%)	
Male	31 (59.6)
Female	21 (40.4)
Birth process, n (%)	
Spontaneous	14 (26.9)
SC	38 (73.1)
Birth weight (grams), median (min-max)	1735 (630-3700)
GA (weeks), mean (95% CI)	33.02 (31.87-34.17)
Birth length (cm), mean (95% CI)	42 (40.27-43.73)

CI: confidence interval; GA: gestational age; SC: *sectio caesarea*.

Table 2. Characteristics of study subjects based on risk factors for infection of EONS subjects (n=52).

Variable	n (%)
PROM	
Yes (>24 hours)	5 (9.6)
Yes (<24 hours)	4 (7.7)
Not	43 (82.7)
Mother has fever	
Yes (>38°C)	2 (3.8)
Yes (< 38°C)	3 (5.8)
Not	47 (90.4)
FHR >160 beats/minute	
Yes	4 (7.7)
Not	48 (92.3)
Green amniotic fluid	
Yes	1 (1.9)
Not	51 (98.1)
Asphyxia	
Yes	32 (61.5)
Not	20 (38.5)
Birth weight (grams)	
VLBW (< 1500)	17 (32.7)
LBW (1500-2499)	23 (44.2)
Normal (2500-4000)	12 (23.1)
Prematurity	
Yes (GA <37 weeks)	38 (73.1)
No (GA >37 weeks)	14 (26.9)
Multiple pregnancies	
Yes	25 (48.1)
Not	27 (51.9)
Vaginal discharge on the mother	
Yes	20 (38.5)
Not	32 (61.5)
UTI in untreated mothers	
Yes	9 (17.3)
Not	43 (83.7)

FHR: fetal heart rate; GA: gestational age; LBW: low birth weight; PROM: premature rupture of membranes; UTI: urinary tract infection; VLBW: very low birth weight.

Table 3. Characteristics of study subjects based on clinical signs of EONS (n=52).

Variable	n (%)
Temperature irregularity	
Yes (hyperthermia)	3 (5.8)
Yes (hypothermia)	2 (3.8)
Not	47 (90.4)
Changes in behavior	
Yes (lethargy)	14 (26.9)
Not	38 (73.1)
Changes in tone	
Yes	18 (34.6)
Not	34 (65.4)
Skin disorders	
Yes (icteric)	6 (11.5)
Yes (cyanosis)	9 (17.3)
Yes (poor peripheral perfusion)	1 (1.9)
Not	36 (69.2)
Gastrointestinal problems	
Yes (bloating)	5 (9.6)
Yes (vomiting)	3 (5.8)
Not	44 (84.6)
Cardiovascular problems	
Yes (respiratory distress)	47 (90.4)
Not	5 (9.6)
Metabolic problems	
Yes (hyperglycemia)	4 (7.7)
Yes (hypoglycemia)	3 (5.8)
Not	45 (86.5)

variables (p=0.000; 95% CI: 0.001-0.004). This meant that every 1 ng/L increase in presepsin levels was associated with an increase in procalcitonin level of 0.002 ng/mL. In addition to presepsin levels, birth weight was also independently affected procalcitonin levels. This meant that every 1 gr increase in birth weight was associated with a decrease in procalcitonin levels of 0.001 ng/mL.

Discussion

The number of male subjects in this study was more than that of female. Male dominance in neonatal sepsis suggests a possible sex-linked factor in host susceptibility (23,24), which is also supported by another study that obtained the same result (25). Vertical transmission of bacteria from mother to fetus during the antenatal and intra-natal periods can also cause EONS.(26-29) In this study, there were 73.1% of EONS subjects that were born prematurely and the lowest neonatal weight in this study was 630 grams. This might have relation to the occurrence of EONS, since premature and LBW are risk factors for neonatal sepsis.(5,30) Increasing the risk of infection later born to eventually become neonatal sepsis lack of immunoglobulin G (IgG) antibodies causes premature neonates to have an immature immune system.(31,32)

The median value of presepsin levels in this study was 108.33 ng/L, with minimum presepsin level was 71.43 ng/L and maximum level was 1287.76 ng/L. There is higher presepsin levels after the onset of sepsis, 315 (120-495) ng/L in septic subjects born at >37 weeks gestation; 390 (120-600) ng/L in septic subjects born prematurely with a birth weight of 1500-2500 grams; 520 (135-750) ng/L in septic subjects born prematurely with a birth weight

Table 4. Characteristics of study subjects based on the laboratory examinations (n=52).

Variable	Value
Leukocyte count (10^3/µL), median (min-max)	12.41 (2.8-26.99)
Neutrophil count (10^3/µL), median (min-max)	6.73 (0.3-20.25)
Platelet count (10^3/µL), median (min-max)	265.5 (29-787)
IT ratio, median (min-max)	0.0885 (0.01-0.38)
Vacuolization and/or toxic granules, n (%)	
Positive	17 (32.7)
Negative	27 (51.9)
Not inspected	8 (15.4)
Blood culture, n (%)	
Escherichia coli	2 (3.8)
Staphylococcus coagulase negative in one specimen	2 (3.8)
Staphylococcus cohnii ss. Urealyticus and *Staphylococcus epidermidis* in two specimens	1 (1.9)
No growth	47 (90.5)
Procalcitonin levels (ng/mL), median (min-max)	0.435 (0.12-9.11)
Presepsin levels (ng/L), median (min-max)	108.33 (71.43-1287.76)
NLR, median (min-max)	1.68 (0.2-7.52)

Table 5. Correlation test results of presepsin levels and NLR with procalcitonin levels.

Variable	Median (min-max)	r	*p*-value
Presepsin levels (ng/L)	108.33 (71.43-1287.76)	0.473	0.000*
NLR	1.68 (0.2-7.52)	0.437	0.001*
Procalcitonin levels (ng/mL)	0.435 (0.12-9.11)		

*statistically significant (p<0.05), compared with procalcitonin level.

of 1000-1500 grams; and 650 (490-1030) ng/L in septic subjects born prematurely with a birth weight of 500-1000 grams. Presepsin levels in neonatal sepsis subjects born at >37 weeks were lower and significantly lower than those born prematurely with low birth weight.(7) Presepsin levels decrease progressively with antibiotics and are beneficial for monitoring response to therapy.(16,24)

NLR is attracting much attention as a new risk factor with the potential for use in the diagnosis of sepsis.(33) The median value of NLR in this study was 1.68, with minimum value was 0.2 and the maximum value was 7.52. Previous study finds a lower median NLR of 1.65 (0.85-7.52) in samples taken after the onset of sepsis.(22)

Procalcitonin synthesis is stimulated by cytokines such as IL-6, IL-1β, and TNF-α, or directly by LPS and downregulated by interferon (IFN)-γ, which are generally produced in response to viral infections. Procalcitonin levels begin to increase four hours after exposure to bacterial endotoxin, peak at six to eight hours and remain increased for at least 24 hours. Procalcitonin half-life estimated 24-30 hours. Procalcitonin levels decrease rapidly after inflammation is resolved.(11,34,35) The lowest procalcitonin levels in this study were 0.12 ng/mL and the levels highest were 9.11 ng/mL.

There is a moderate positive correlation between presepsin levels and procalcitonin in EONS patients with r=0.473, p<0.001. The results of this study following study are conducted on EONS subjects that obtain a moderate positive correlation between presepsin and procalcitonin (r=0.58; p<0.01) (25), and the study on neonatal sepsis subjects in NICU that find a significant positive correlation between presepsin and procalcitonin (r=0.655; p<0.01) (7).

The results of this study indicate that presepsin levels can reflect the state of sepsis. Presepsin release and increased production of procalcitonin by monocytes or macrophages occur after the body's natural response against bacterial infection. A cluster of differentiation 14 activates TLR4 which is a specific proinflammatory signaling cascade to initiate an inflammatory reaction against microorganisms. Presepsin is released after cleavage of the bacterial-LBP CD14-LPS binding on monocytes or macrophages, whereas procalcitonin is produced after monocytes or macrophages release proinflammatory cytokines such as IL-6, IL-1β, and TNF-α or are stimulated directly by bacterial LPS.(12,15)

There is a moderate positive correlation between NLR and procalcitonin in EONS patients with r=0.437, p=0.001. The result of this study is in accordance with previous study that obtained a moderate positive correlation between NLR and procalcitonin in neonatal sepsis after the onset of sepsis (r=0.531; p<0.001).(22)

Based on the results of multivariate analysis using a general linear model, presepsin levels and birth weight

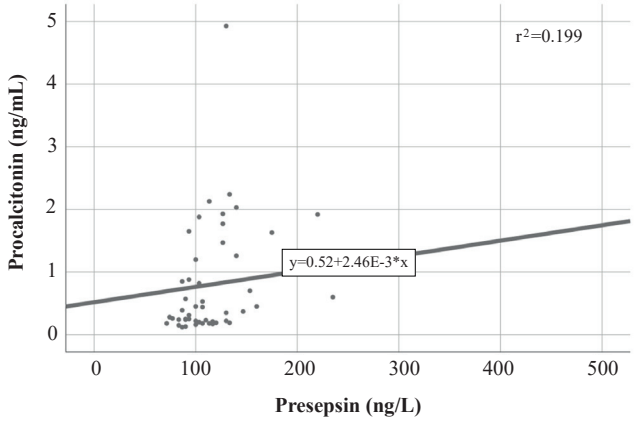

Figure 1. Scatter plot correlation between presepsin and procalcitonin levels.

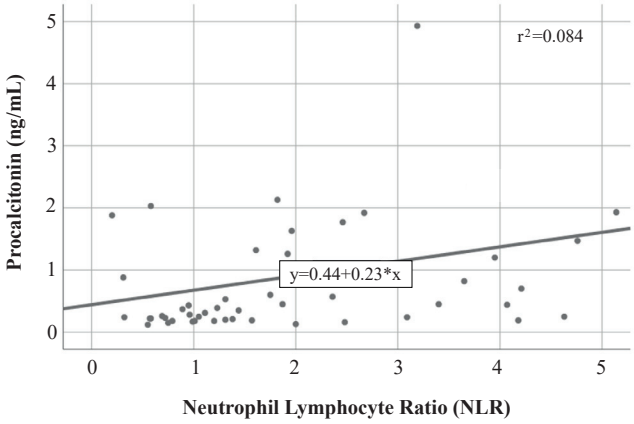

Figure 2. Scatter plot correlation between NLR and procalcitonin levels.

Table 6. Linear regression test results of the relationship between presepsin and procalcitonin levels after controlling for confounding variables.

Variable	Coefficient B	95% CI	*p*-value
Presepsin levels	0.002	0.001 – 0.004	0.000*
Age	0.372	-0.032 – 0.776	0.070
Gender	0.344	-0.421 – 1.108	0.370
Birth weight	-0.001	-0.002 - -3.543	0.001*
Birth process	-0.412	-1.067 – 0.292	0.292
PROM	0.233	-0.310 – 0.776	0.392
NLR	0.114	-0.093 - 0.322	0.273

CI: confidence interval; PROM: premature rupture of membranes. *statistically significant (p<0.05).

was shown to independently affect procalcitonin levels. Other studies also show similar results, and obtain that procalcitonin levels are significantly higher in neonatal sepsis subjects with lower birth weight and following the statement that procalcitonin levels are affected by birth weight.(7,11)

Because the research location was carried out in a referral hospital (type A), the study sample population may be different from the patient population in type B and C hospitals, so it has a limited scope of generalization only at referral hospitals. Further research needs to be done with a larger sample size and wider coverage (multicenter) or several types of hospitals to produce a wider generalization coverage of research results.

Conclusion

Results of this study show a moderately significant correlation between presepsin and NLR with procalcitonin levels in EONS patients, suggesting that presepsin levels, NLR, and procalcitonin levels are potential candidates for EONS biomarkers.

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Authors Contribution

NPSSP, NKM, MS, AANS, and IWGAEP were involved in planning and supervising the work. NPSSP, NKM, and INW, performed the measurements, processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. NPSSP, IWGAEP, and AAWL performed the calculations and statistical analysis. NPSSP, NKM, MS, AAWL, INW, and NNM aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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