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Increased IL-31 expression in serum and tissue protein in prurigo nodularis

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Abstract

Background: Prurigo nodularis (PN) is a chronic pruritic skin disease which can greatly impact patients' quality of life. Moreover, the pathogenesis remains unclear, making it a difficult-to-treat condition.

Aims: To investigate the expression of interleukin-31 (IL-31) in serum and skin biopsy specimens of PN patients and healthy subjects and identify its possible correlation to disease severity and itch intensity.

Methods: Patients with PN and healthy volunteers were recruited for the study. Expression levels of IL-31 were measured by enzyme-linked immunosorbent assay and immunohistochemistry. Baseline characteristics, disease activity, itch intensity, and related laboratory results were collected.

Results: Forty-three PN patients and 31 healthy subjects participated in our study. The PN patients had significantly higher mean serum IL-31 levels than the healthy subjects (52.9 ± 18.2 *versus* 36.3 ± 10.7 pg/ml, $p < 0.001$). Epidermal and dermal PN lesions also exhibited significantly higher IL-31 expression compared with the healthy skin ($p < 0.001$ and $p = 0.01$, respectively). However, there was no significant difference in serum or lesional expression of IL-31 by disease severity or itch intensity.

Conclusion: Increased IL-31 expression in serum and PN lesions suggests that IL-31 has a potential role in the pathogenesis of PN.

Keywords: interleukin-31, pathogenesis, prurigo nodularis

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Introduction

Prurigo nodularis (PN) is a chronic skin condition characterized by firm, intensely pruritic papulonodular eruptions that mostly occur on extensor surfaces of the extremities with a symmetric distribution.[1] Because PN results in intractable pruritus and skin lesions that are predominantly on exposed areas, it may greatly impair patients' quality of life.[2] The exact pathogenesis of PN remains unknown, but interaction between the central/peripheral nervous system and cutaneous inflammation is thought to play a crucial role, leading to a repetitive itch-scratch cycle.[1,3] Pruritus is caused when inflammatory cells in the skin release various substances including tryptase, eosinophil cationic protein, histamine, prostaglandins, nerve growth factor, substance P, calcitonin gene-related peptide, and interleukin (IL)-31.[3]

IL-31, a member of the gp130/IL-6 family of cytokines, is thought to play a significant role in the induction of pruritus in various chronic inflammatory skin conditions including PN.[4,5] IL-31 is mainly produced by activated CD4+ Th2 cells and binds to its heterodimeric receptors, IL-31 receptor alpha (IL-31RA) and oncostatin M receptor beta (OSMRβ), and is expressed on epithelial cells including keratinocytes.[4] The signaling pathways JAK/STAT (Janus-activated kinase/signal transducer and activator of transcription),

PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase), and MAPK (mitogen-activated protein kinase) are subsequently activated, leading to the release of chemokines and proinflammatory cytokines including itch mediators.[6,7] Overexpression of IL-31 messenger RNA (mRNA) in PN lesions compared with healthy skin or non-lesional skin has been reported,[5,8] but immunohistochemistry has demonstrated decreased expression of IL-31 in PN lesions compared with other skin conditions such as psoriasis, mycosis fungoides, Sézary syndrome, and perilesional skin.[9] As there have yet been no studies focusing on serum IL-31 analysis in PN patients, we compared IL-31 expression at the protein level in serum and skin biopsy specimens from patients with PN with that in those taken from healthy subjects in order to better understand the role of IL-31 in PN.

Methods

Participants

This is a cross-sectional, analytical study conducted at Khon Kaen University's Srinagarind Hospital in Thailand. The study was approved by the Khon Kaen University ethics committee (HE601361), and written informed consent was obtained from all participants before enrollment in the study. Patients diagnosed with PN between October 2017 and December 2019 were included. Diagnosis was based on clinical examination and histological confirmation, and patients with systemic conditions, such as anemia, chronic kidney disease, liver disease, thyroid disease, malignancy, and neuropsychiatric disorders, were excluded. Other exclusion criteria included use of any systemic steroids, immunosuppressive drugs, or phototherapy within 3 months prior to participating in the study. The healthy group consisted of healthy adults undergoing plastic surgical reconstructive or excisional procedures at Srinagarind Hospital with no history of pruritic symptoms or co-morbid diseases including malignancy.

Disease activity and itching severity assessment

Disease severity was evaluated using the Prurigo Nodularis Area and Severity Index (PNASI),[10] which assesses erythema, induration, and area involvement. Erythema and induration scores were rated as 1 = mild, 2 = moderate, or 3 = severe. Area involvement was graded as 1 = 1–9% of body surface area (BSA), 2 = 10–29%, 3 = 30–49%, 4 = 50–69%, 5 = 70–89%, or 6 = 90–100%. The total score was the summation of erythema, induration, and area involvement scores and ranged from 1 to 12 points. Total scores were classified into three levels: 0–4 = mild, 5–8 = moderate, and 9–12 = severe.

Itching severity was assessed using an 11-point numeric rating scale (NRS). The NRS-11 evaluates average intensity of pruritus ranging from 0 (no itch) to 10 (worst imaginable itch). Scores were classified into three groups as follows: 0–3 = mild, 4–7 = moderate, and 8–10 = severe.

Baseline characteristics and laboratory parameters

Baseline characteristics, including age, sex, duration of disease, disease severity, itch intensity, laboratory results, and serum IL-31 levels, of all participants were recorded. Complete blood count, serum creatinine, and alkaline phosphatase (ALP) were obtained for all participants. Serum thyroid-stimulating hormone (TSH) was tested only in PN patients. All laboratory tests were performed at the hospital's central laboratory. Serum specimens were obtained from all participants. Skin biopsy specimens were obtained from all PN patients, but only from 10 healthy subjects.

Analysis of serum IL-31 levels

Serum specimens were collected and stored at −80°C. Concentrations of IL-31 in serum were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) with standard kits according to the manufacturer's instructions (Human IL-31 ELISA Kit, Abcam, Cambridge, MA, USA).

Skin biopsy and immunohistochemistry

Four millimeter punch biopsies were taken from the lesional skin of 43 PN patients and 10 healthy subjects. Formalin-fixed, paraffin-embedded, 4-µm-thick biopsies from lesional skin were deparaffinized and rehydrated to be examined for IL-31 immunoreactivity. Anti-IL-31 (ab102750, Abcam, Cambridge, MA, USA, 1:50 dilution) immunohistochemistry was automatically performed with the Ventana BenchMark XT® UltraView DAB detection kit (v3) with incubation

with antibody for a period of 32 min. All samples were then counterstained with hematoxylin II (Ventana, 790–2208) and bluing reagent (Ventana, 760–2037). Appropriate positive controls were used as recommended by the manufacturer. All immunohistochemically stained slides were scanned using an Aperio Slide Scanner (Leica, Germany).

Quantification of immunohistochemical staining

Five different fields of epidermis, including the edges of both sides, the center of the tissue sections, and the area between the edge and the centers of both sides, were captured with an Aperio ImageScope (20× objective magnification). Image analysis was performed on a selected area (a 200 × 100 pixel rectangle) of each photograph using ImageJ software as described previously.[11] The three areas of the upper dermis with the highest density of inflammatory cells (40× objective magnification) per visual field were selected, and IL-31-positive and negative cells were counted in each area. Dermal immunopositive cells were expressed as a percentage of total cells. Data were presented as the mean average epidermal intensity and the mean percentage of positive cells in the dermis. All samples were examined by two independent examiners.

Sample size calculation

As there has been no previous study of serum IL-31 in PN, we referred to a study conducted in patients with atopic dermatitis, which has a similar pathogenesis to that of PN, for sample size calculation.[12] The atopic dermatitis group and healthy control group had serum IL-31 of 43,142.8 (SD, 66,981.6) and 7881.8 (SD, 1842.7) pg/ml, respectively. For a two-group comparison, the required sample size for a confidence of 95% and power of 80% was 29 patients in each group.

Statistical analysis

Statistical analysis was performed using STATA version 10.1 (College Station, Texas, USA). Parametric data were presented as mean ± standard deviation (SD). Non-parametric data were presented as median with interquartile range (IQR). An independent *t*-test and Wilcoxon rank-sum test were used to compare differences between two groups, and a one-way analysis of variance (ANOVA) was used to compare among more than two groups. Variables associated with IL-31 were identified using a logistic regression model. The significant variables selected from univariate analysis were subjected to subsequent multivariate analysis. *p*-values below 0.05 were considered statistically significant.

Results

Patient characteristics

There were 43 PN patients and 31 healthy subjects who participated in this study. The median duration of disease in PN patients was 24 months. Five (11.63%) of the 43 PN patients had a history of atopy, three with allergic rhinitis and two with asthma. Five patients (11.63%) had diabetes. The median range of TSH in the PN group was 2.0 (1.2, 2.9) mIU/L. The PN group differed from the healthy groups significantly in terms of age, total monocyte count, and total eosinophil count (Table 1).

Serum IL-31 levels in the PN and healthy groups

Serum IL-31 levels were significantly higher in the PN group than in the healthy group (52.9 ± 18.2 *versus* 36.3 ± 10.7 pg/ml, $p < 0.001$; Figure 1). Of the 43 PN patients, 10 had mild, 28 had moderate, and 5 had severe PN based on their PNASI scores. There was no significant difference in IL-31 levels among these groups ($p = 0.79$; Table 2). Itch intensity was evaluated according to the NRS-11. Two patients had mild, 11 had moderate, and 30 had severe itch. No significant difference in IL-31 levels among these groups was detected ($p = 0.90$; Table 3).

Epidermal expression of IL-31 in the PN and healthy groups

All samples in both groups showed positive IL-31 immunoreactivity throughout the entire epidermis (Figure 2(a)). Epidermal IL-31 expression as measured using ImageJ was significantly enhanced in the PN group compared with healthy subjects [174.6 ± 20.1 *versus* 130.5 ± 16.3 arbitrary unit (AU), $p < 0.001$; Figure 2(b)]. No significant difference in epidermal IL-31 expression by disease severity or itch intensity was detected ($p = 0.33$; Table 2 and $p = 0.95$; Table 3, respectively).

Table 1. Baseline characteristics and laboratory results of prurigo nodularis (PN) patients and healthy subjects.

Factors	PN patients $n = 43$	Healthy subjects $n = 31$	*p*-values
Age (years): mean ± SD	53 ± 18	44 ± 18	0.03
Female sex, *n* (%)	22 (48.8)	20 (60.6)	0.49
Hemoglobin (g/dl): mean ± SD	13.3 ± 1.3	13.4 ± 1.2	0.70
White blood cell (cells/mm^3): mean ± SD	7765.8 ± 2400.2	7198.2 ± 1875.4	0.30
Total neutrophil count (cells/mm^3): mean ± SD	4540.2 ± 2101.1	4297.6 ± 1631.9	0.60
Total lymphocyte count (cells/mm^3): mean ± SD	2101.2 ± 1096.5	2113.5 ± 618.3	0.95
Total monocyte count (cells/mm^3): median (IQR)	573.4 (427.5, 773.1)	475.2 (401.5, 559.6)	0.03
Total eosinophil count (cells/mm^3): median (IQR)	314.8 (144.8, 527.4)	143.0 (73.9, 283.9)	0.02
Total basophil count (cells/mm^3): median (IQR)	41.9 (32.6, 56.8)	40.9 (29.7, 58.9)	0.75
Serum creatinine (mg/dl): mean ± SD	0.9 ± 0.3	0.8 ± 0.2	0.06
Serum alkaline phosphatase (U/L): mean ± SD	77.1 ± 26.0	71.1 ± 24.1	0.39

IQR, interquartile range; SD, standard deviation.

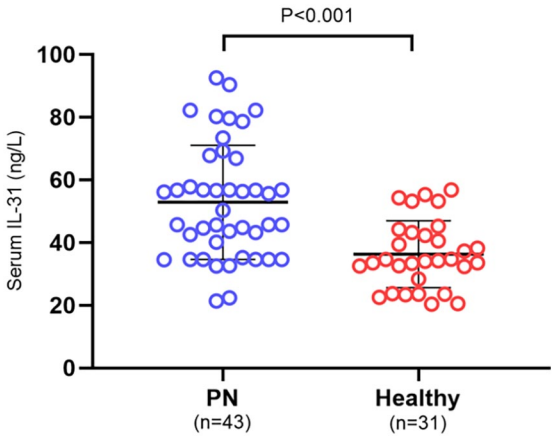

Figure 1. Comparison of serum IL-31 levels between PN patients and healthy subjects. Serum IL-31 levels were significantly higher in PN patients than in healthy subjects.

Dermal expression of IL-31 in the PN and healthy groups

Strong cytoplasmic staining of inflammatory cells in the dermis was considered to account for the immunoreactivity for IL-31 (Figure 2(a)). The percentage of IL-31-positive cells in the PN group was significantly higher than that found in the control group [69.2% ± 16.5% *versus* 53.2% ± 15.3%, $p = 0.01$; Figure 2(c)]. However, no significant difference in the percentage of immunopositive cells by disease severity or itch intensity was detected ($p = 0.17$; Table 2 and $p = 0.91$; Table 3, respectively).

IL-31 expression in PN patients with history of atopy and those without

We compared serum and lesional expression of IL-31 in the 5 PN patients with a history of atopy and the 38 patients without atopy. No differences were found in terms of serum, epidermal, or dermal expression of IL-31 ($p = 0.50$, 0.61, and 0.92, respectively; Table 4).

Based on multivariate logistic regression analysis, only PN was an independent factor associated with IL-31, as shown in Table 5.

Discussion

Although the pathogenesis of PN is not yet completely understood, inflammatory cells and various itch mediators and cytokines play important

Table 2. IL-31 levels and expression in PN patients according to disease severity.

PNASI scores	Mild ($n=10$)	Moderate ($n=28$)	Severe ($n=5$)	*p*-values
Serum IL-31 (pg/ml): mean ± SD	56.5 ± 16.5	50.3 ± 18.2	60.0 ± 22.1	0.79
IL-31 epidermal intensity (AU): mean ± SD	176.5 ± 15.2	175.1 ± 20.7	165.9 ± 29.8	0.33
IL-31 percentage of positive cells in dermis (%): mean ± SD	71.2 ± 9.6	68.1 ± 18.9	73.8 ± 15.4	0.17

AU, arbitrary unit; IL, interleukin; PN, prurigo nodularis; PNASI, Prurigo Nodularis Area and Severity Index; SD, standard deviation.

Table 3. IL-31 levels and expression in PN patients according to itch intensity.

NRS-11	Mild ($n=2$)	Moderate ($n=11$)	Severe ($n=30$)	*p*-values
Serum IL-31 (pg/ml): mean ± SD	45.2 ± 17.8	53.2 ± 16.9	53.2 ± 19.2	0.90
IL-31 epidermal intensity (AU): mean ± SD	170.2 ± 24.9	166.3 ± 20.1	177.7 ± 19.8	0.95
IL-31 percentage of positive cells in dermis (%): mean ± SD	80.2 ± 13.1	65.2 ± 15.2	69.5 ± 17.1	0.91

AU, arbitrary unit; IL, interleukin; PN, prurigo nodularis; NRS-11, 11-point numeric rating scale; SD, standard deviation.

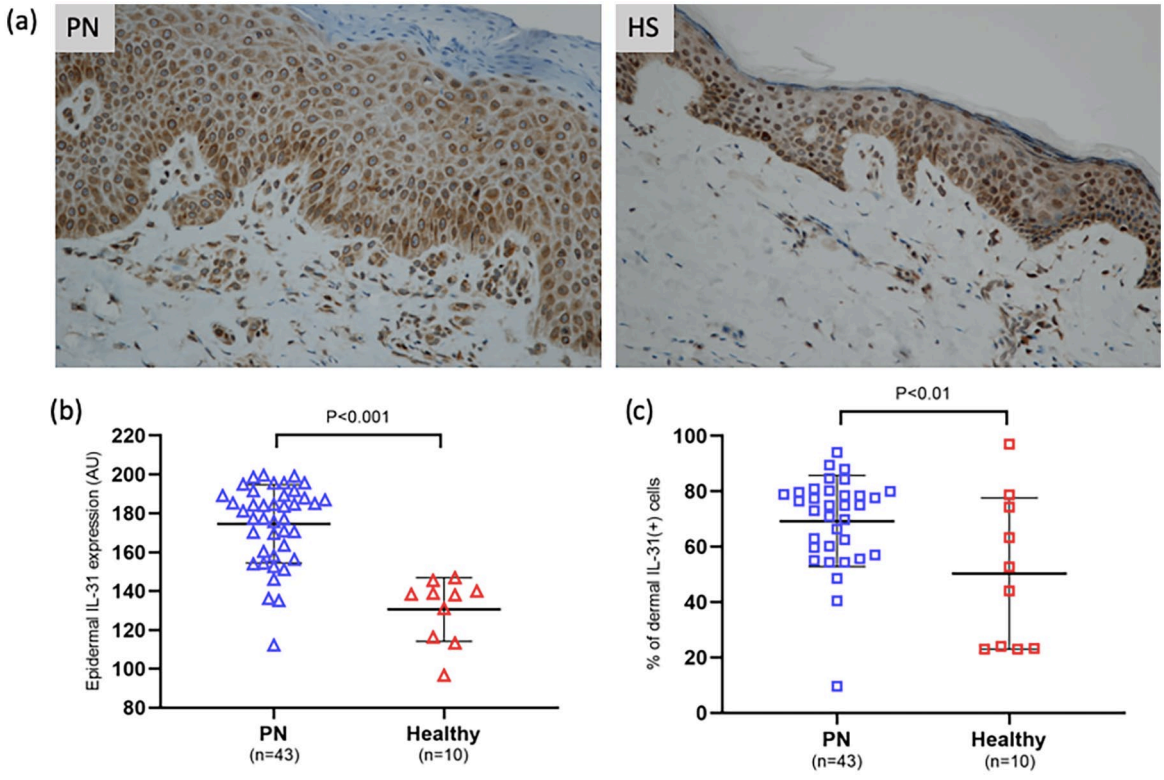

Figure 2. Cutaneous expression of IL-31 by immunohistochemistry staining from a prurigo nodularis (PN) patient and a healthy subject (HS): (a) (original magnification ×400). Comparison of IL-31 expression in the epidermis (b) and dermis (c) between PN patients and healthy subjects, evaluated by immunohistochemistry. Epidermal and dermal expression of IL-31 were significantly higher in PN patients than in healthy subjects.

Table 4. IL-31 levels and expression in PN with atopy and PN without atopy.

Variables	PN with atopy ($n=5$)	PN without atopy ($n=38$)	*p*-values
Serum IL-31 (pg/ml): mean ± SD	58.1 ± 9.9	52.2 ± 10.7	0.50
IL-31 epidermal intensity (AU): mean ± SD	170.2 ± 11.0	175.2 ± 3.3	0.61
IL-31 percentage of positive cells in dermis (%): mean ± SD	69.9 ± 17.3	69.1 ± 16.6	0.92

AU, arbitrary unit; IL, interleukin; PN, prurigo nodularis; SD, standard deviation.

Table 5. Factors associated with interleukin-31 levels in patients with PN and healthy subjects by multivariate linear regression analysis.

Factors	Multivariate coefficient	95% CI	*p*-values
Serum IL-31			
Presence of PN	15.784	7.655, 23.913	<0.001
Age	0.132	−0.094, 0.358	0.252
Monocyte count	−0.002	−0.011, 0.006	0.612
Eosinophil count	0.001	−0.010, 0.011	0.926
Creatinine	0.773	−13.310, 14.856	0.914
Epidermal intensity			
Presence of PN	42.283	28.660, 55.911	<0.001
Age	0.178	−0.170, 0.528	0.316
Monocyte count	−0.006	−0.026, 0.014	0.584
Eosinophil count	0.004	−0.009, 0.017	0.540
Creatinine	−9.471	−27.316, 8.373	0.298
Percentage of dermal IL31+ cells			
Presence of PN	17.112	4.238, 29.986	0.009
Age	0.102	−0.261, 0.465	0.582
Monocyte count	−0.014	−0.034, 0.007	0.192
Eosinophil count	−0.010	−0.022, 0.002	0.104
Creatinine	−1.665	−17.989, 14.658	0.842

CI, confidence interval; IL, interleukin; PN, prurigo nodularis.

roles in its development. IL-31 is a pruritogenic cytokine involved in non-histaminergic itch pathways.[13] Recent studies have shown that IL-31 plays a role in chronic pruritic inflammatory skin diseases including atopic dermatitis,[14–16] psoriasis,[17,18] and PN.[5] However, no studies have

examined serum IL-31 in PN patients. This study showed that patients with PN had significantly higher serum IL-31 levels than healthy subjects. Moreover, epidermal and dermal expressions of IL-31 in the lesional skin of PN patients were also significantly higher than in healthy subjects. Previous studies have found higher IL-31 mRNA expression in PN lesions than in healthy skin.[5,8] Recently, a study found that nemolizumab, a humanized monoclonal antibody targeting the IL-31 receptor that inhibits the binding of IL-31 to its receptor, significantly reduced pruritus and the severity of skin lesions in moderate to severe PN patients compared with a placebo.[19] Taken together, this implies that IL-31 plays a role in the pathogenesis of PN.

A previous study of PN examining immunostaining of skin biopsy specimens found decreased expression of IL-31 in lesions compared with other skin conditions including psoriasis, mycosis fungoides, Sézary syndrome, and perilesional skin.[9] One study showed that dermal expression of IL-31 in PN was significantly correlated with itch intensity.[20] Our study found that IL-31 expression was significantly enhanced in the epidermal and dermal lesions of PN patients compared with the healthy group, although its expression was not significantly correlated with disease severity or with itch intensity. This might be due to the limited sample size, which may have led to non-normally distributed disease severity and itch intensity in the PN group. It may also have been due to the subjective assessment of itch intensity. A large clinical trial should be conducted to investigate further.

PN is commonly associated with history of atopy such as atopic dermatitis, asthma, and allergic rhinitis. This study found only five patients who had allergic rhinitis or asthma, which may have been due to the high prevalence of late-onset non-atopic PN (mean age, 53 ± 18 years) in our population. Previous studies found that serum IL-31 levels were significantly elevated in patients with asthma and allergic rhinitis compared with controls.[21,22] However, this study showed no significant differences in IL-31 levels between PN patients with a history of atopy and those without. This might be due to small percentage of PN patients with atopy in our study.

Our previous studies demonstrated that serum IL-31 levels were significantly higher in patients with chronic pruritus of unknown origin, chronic spontaneous urticaria, or psoriasis with pruritic symptoms compared with healthy subjects.[23,24] Median serum IL-31 in patients with chronic pruritus of unknown origin was about 3.7 times higher than in healthy controls (127.3 *versus* 34.4 pg/ml), and mean serum IL-31 in patients with chronic spontaneous urticaria and those with psoriasis was about 7 times higher (252.4 ± 115.5 *versus* 36.3 ± 10.7 pg/ml, $p < 0.001$) and 3 times higher (121.4 ± 16.6 *versus* 36.3 ± 10.7 pg/ml, $p < 0.001$) than in healthy controls, respectively.[23,24] This study found that mean serum IL-31 in the PN group was only 1.5 times higher than in the healthy group. This might be due to the inhibitory effect of other pruritic mediators and cytokines on circulating IL-31 levels, leading to lower levels of serum IL-31 in PN than in other pruritic conditions. A previous clinical trial showed that nemolizumab significantly improved symptoms of pruritus and severity of disease in moderate to severe PN patients,[19] and our study found increased IL-31 expression in serum and tissue protein in PN patients. However, no studies have examined how levels of serum IL-31 change during the course of nemolizumab treatment. This data might be helpful in determining whether serum IL-31 levels can be used as a potential biomarker to predict clinical response to nemolizumab treatment in patients with PN.

This study had some limitations. First, only IL-31 was measured. Second, the sample size was relatively small and the number of patients with each level of disease severity and itch intensity varied. Further studies with other pruritic mediators and cytokines, and follow-up research examining complete remission after treatment, are required to determine the role of IL-31 and other pruritic mediators in the pathogenesis of PN.

Conclusion

Serum IL-31 levels were significantly higher in PN patients than in healthy subjects. Epidermal and dermal expressions of IL-31 in PN lesional skin were also significantly higher than in healthy subjects. However, there was no significant difference in the expression of IL-31 in serum or lesional skin by disease severity or itch intensity.

Declarations

Ethics approval and consent to participate

This study was approved by the Khon Kaen University ethics committee (HE601361), according to the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment in the study.

Consent for publication

All authors approved the final manuscript.

Author contributions

Suteeraporn Chaowattanapanit: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Validation; Visualization; Writing – original draft; Writing – review & editing.
Rachot Wongjirattikarn: Data curation; Formal analysis; Investigation; Validation; Visualization; Writing – original draft.
Nipon Chaisuriya: Conceptualization; Investigation; Methodology; Validation; Visualization; Writing – original draft.
Piti Ungarreevittaya: Conceptualization; Investigation; Methodology; Validation; Visualization; Writing – original draft.
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Kengkart Winaikosol: Conceptualization; Investigation; Writing – review & editing.
Charoen Choonhakarn: Conceptualization; Investigation; Writing – review & editing.
Narachai Julanon: Conceptualization; Investigation; Writing – review & editing.
Kanin Salao: Conceptualization; Investigation; Writing – review & editing.

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Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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