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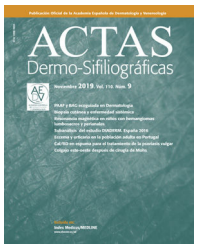

ORIGINAL ARTICLE

Plasma Steroids and Endocannabinoids Used as Biomarkers to Assess the Pruritus Severity of Patients With Prurigo Nodularis

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KEYWORDS
Prurigo nodularis;
Plasma biomarkers;
Endocrine systems;
Pruritus severity

Abstract
Background: Prurigo nodularis (PN) as an extremely pruritic and hyperplastic chronic dermatosis induces psychologically and physiologically stressful responses. PN-induced responses in the hypothalamic–pituitary–adrenal (HPA), hypothalamic–pituitary–gonadal (HPG) axes and endocannabinoid system (ECS) are abnormal. Extant studies on the PN's pathogenesis mostly focused on the PN-psychological responses. To date, the PN's physiological responses remain not been fully uncovered yet.
Objectives: To investigate the PN-induced physiological responses via the levels of five steroids and two endocannabinoids combined with their ratios in plasma and examine the association between the psychological and physiological responses.
Materials and methods: Thirty-six patients with PN, 36 age- and gender-matched healthy controls were recruited. The PN's psychological symptoms including pruritus severity, pain and life quality were measured with the visual analog scale, the prurigo score index, numerical rating scale, verbal rating scale and dermatology life quality index. Their concentrations of steroids and endocannabinoids were determined with liquid chromatography-tandem mass spectrometry.

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Results: Compared to controls, the PN patients showed lower plasma levels in cortisol, cortisone, *N*-arachidonoyl-ethanolamine (AEA), and the ratio of DHEA to 1-arachydonoyl glycerol (1-AG), which negatively moderately and over correlated with PN's symptoms, especially with the pruritus severity. Additionally, the PN patients exhibited higher levels in the ratios of testosterone and 1-AG to cortisol, which positively moderately and over correlated with pruritus severity. Thus, the seven biomarkers would be sensitive and reliable biomarkers for assessing the pruritus severity of PN because they met the screening criteria that the biomarkers show intergroup differences and showed moderate or over correlation with the pruritus severity of PN.
Conclusions: To the best of our knowledge, this is the first study exploring PN-induced physiological responses. The findings suggest that alterations in these three endocrine systems may lead to new insights to psychological mechanisms and responses to prurigo nodularis.

PALABRAS CLAVE
Prúrigo nodular;
Biomarcadores plasmáticos;
Sistema endocrino;
Gravedad del prurito

Esteroides y endocannabinoides plasmáticos utilizados como biomarcadores para evaluar la gravedad del prurito de los pacientes con prúrigo nodular

Resumen

Antecedentes: El prúrigo nodular (PN), una dermatosis crónica pruriginosa e hiperplásica, induce respuestas psicológica y fisiológicamente estresantes. Las respuestas inducidas por PN en los ejes hipotalámico-pituitario-adrenal (HPA) e hipotalámico-pituitario-gonadal (HPG) y en el sistema endocannabinoide (SEC) son anómalas en cierta medida. Los estudios sobre la patogenia de PN se centran principalmente en las respuestas psicológicas del mismo. Hasta la fecha no se han revelado plenamente las respuestas fisiológicas.
Objetivos: Investigar las respuestas fisiológicas inducidas por PN a través de los niveles de cinco esteroides y dos endocannabinoides junto con sus ratios a nivel plasmático, y examinar la asociación entre las respuestas psicológicas y fisiológicas.
Materiales y métodos: Se seleccionaron treinta y seis pacientes con PN, y 36 controles sanos equiparados por sexo y edad. Se midieron los síntomas psicológicos de PN incluyendo la gravedad y dolor del prurito y la calidad del sueño con la escala visual analógica, el índice de puntuación del prúrigo, la escala de calificación numérica, la escala de calificación verbal, y el índice de calidad de vida dermatológica. Se determinaron las concentraciones de esteroides y endocannabinoides mediante cromatografía de líquidos-espectometría de masas en tándem.
Resultados: En comparación con los controles, los pacientes de PN reflejaron menores niveles plasmáticos de cortisol, cortisona, *N*-araquidonoiletanolamina (AEA), y el ratio DHEA con respecto a 1-araquidonol glicerol (1-AG), que se correlacionaron de manera negativamente moderada y estrecha con los síntomas de PN, especialmente con la gravedad del prurito. Además, los pacientes de PN mostraron niveles mayores de los ratios de testosterona y 1-AG con respecto a cortisol, que se correlacionaron de manera positivamente moderada y estrecha con la gravedad del prurito. Por ello, los siete biomarcadores serían sensibles y fiables a la hora de evaluar la gravedad del prurito de PN, dado que cumplen los criterios de cribado, muestran diferencias entre los grupos, y reflejan una correlación moderada o estrecha con la gravedad del prurito de PN.
Conclusiones: A nuestro entender, el nuestro es el primer estudio que explora las respuestas fisiológicas inducidas por PN. Los hallazgos sugieren que las alteraciones en estos tres sistemas endocrinos pueden conducir a nuevas percepciones sobre los mecanismos y respuestas psicológicas al prúrigo nodular.

Introduction

Prurigo nodularis (PN) as a chronic disease would result in an individual's psychological and physiological responses.[1,2] Existing studies have investigated the psychological responses related to PN,[2–7] such as emotional and psychosomatic responses. For instance, numerous studies had demonstrated that PN patients showed higher prevalence psychological symptoms, e.g. anxiety and depression, than healthy controls[2–5] and the patients with psoriasis vulgaris.[8–10] Moreover, previous studies concerning the PN-related physiological aspects mainly focused on dermatosis-induced immune such as excessive releasing of cutaneous nerve fibers and neuropeptides.[11,12] However,

the PN-related physiological responses to endocrine systems still remain poorly understood. On the other hand, Schneider et al. suggested that a psychological assessment in combination with objective physiological index should be included in clinical management of the PN patients[7] rather than self-report psychological questionnaires.[3,7,13–17] The intensity of pruritus is effectively evaluated by the visual analog scale (VAS)[18,19] and the prurigo score (PRUNOSI) recently developed.[20] The intensity of pain is measured with the numerical rating scale (NRS) and verbal rating scale (VRS).[18,21] Additionally, previous studies also concerned for the patients' health status itching and scratching in PN would remarkably degrade patients' quality of life.[22] The patients' quality life is measured with dermatology life quality index (DLQI).[23] Although they offered an easy and inexpensive possibility to gain an insight into patients' stress responses, self-report psychological measures are lack of objectiveness, resulting in an over-evaluated on the PN-induced stressful responses. Therefore, it is urgent to investigate the PN-related physiological responses for developing objective biomarkers to fully assess stressful responses in PN patients.

Although there has been no empirical study investigating PN's physiological responses, some inspirations can be obtained from similar chronic dermatology such as chronic urticarial. Patients with chronic urticaria showed a lower basal contents of cortisol and dehydroepiandrosterone sulfate that is a metabolite of dehydroepiandrosterone (DHEA)[24] than healthy subjects,[25,26] which were attributed to the fact that the activity of the hypothalamo–pituitary–adrenal (HPA) axis was fatigue under long-term chronic stress. However, a single and dual-steroid's concentration cannot fully reflect the activity of the HPA axis and a single endocrine system (i.e., the HPA axis) cannot comprehensively illustrate the alterations in other stress-sensitive neuroendocrine systems related to skin disease because psychosomatic factors related to skin disease could trigger the disease and to influence the disease's course.[27,28]

Alternatively, the use of multiple-steroids' concentrations would comprehensively assess the HPA activity and other neuroendocrine systems, such as the hypothalamo–pituitary–gonadal (HPG) axis and endogenous cannabinoid system (ECS). The three systems are the stress-sensitive neuroendocrine systems in human beings.[29–31] Traditionally, the endogenous steroids and endocannabinoids that are secreted by the three systems and the secretion of which is regulated by the three systems were used as biomarkers to reflect the activities of the three systems.[32–35] In particular, the steroids and endocannabinoids also exert multifarious physiological and psychological effects on bear stress.[36–40] Therefore, these biomarkers are reliable in assessing the PN-related physiologically stressful responses caused by PN-associated psychological changes[1,2] and might be quite objective in assessing the PN-related stressful responses. For instance, both cortisol and cortisone contents were commonly used to assess the activity of the HPA axis.[29,41] In parallel, simultaneous use of DHEA, testosterone and progesterone as important sex steroids were recommended to assess the activity of the HPG axis.[42,43] The concentrations of both N-arachidonoyl-ethanolamine (AEA) and 1-arachydonoyl glycerol (1-AG) were used to evaluate the ECS activity.[44–46]

Moreover, the three neuroendocrine systems interact with each other via endogenous compounds that they secreted.[32–35] The ratios between the concentrations of endogenous steroids and endocannabinoids can be potentially used as novel biomarkers to assess the interactions within one endocrine system and between the three systems[47–51] because of their biological significances. The ratio of cortisone to cortisol was used to assess the interaction within the HPA axis, i.e., the interconversion between cortisol and cortisone under the enzyme catalysis.[47] The AEA/1-AG ratio was used to evaluate the interaction within the ECS.[51] The ratios of testosterone and DHEA to cortisol reflect the interaction between the HPA and HPG axes.[48–50] The ratios of AEA and 1-AG to cortisol, the ratios of testosterone to AEA and 1-AG, DHEA/AEA ratio and DHEA/1-AG ratio might be used to reflect the interactions of the HPA and HPG axes with ECS. More recently, our research has shown that the contents of endogenous steroids and endocannabinoids in combination with their ratios were more sensitive and reliable to comprehensively evaluate the activities of the HPA and HPG axes and ECS as well as the interactions within, between and among the three neuroendocrine systems in early acne.[52] Therefore, the present study will evaluate the differences between patients with PN and healthy controls in multiple biomarkers including plasma cortisol, cortisone, testosterone, progesterone, DHEA, AEA, 1-AG and their ratios. The use of the multiple biomarkers will be helpful for screening the sensitive and reliable biomarkers to distinguish the PN patients from healthy controls and for understanding association of PN-related responses with interactions within one endocrine system and between multiple endocrine systems. And then, we also explore the relationships of the physiological responses assessed by objective endocrine biomarkers with the PN's psychological responses evaluated by self-report questionnaires. This will be helpful for screening the sensitive physiological biomarkers that could accurately assess the pruritus severity of PN.

Methods

Participants and Study Design

Thirty-six patients with PN and 36 age- and gender-matched healthy controls were recruited for this study from Peking University Shenzhen Hospital. The inclusion criteria of PN patients were as follows: (1) age >18 years, (2) onset of skin of duration and the length of the systems being present and the number of lesions almost same and (3) no history of taking steroids or immunosuppressant or any other medication within 4 weeks prior to participating in the study. The exclusion criteria included: (1) patients with any physical diseases (e.g., tumor, organic brain diseases, thyroid disease and autoimmune diseases) and (2) patients with a history of family psychiatric disorders. In addition, demographic information (e.g., age, gender and BMI) from participants were recorded.

Patients with PN were asked to record their current PN's symptoms, such as pruritus and pain, via a questionnaire

including four categories, the prurigo score[22] (PRUNOSI ranging 0–3), the visual analog scale[53] (VAS, a graphic tool with a 100-mm horizontal line with the left end marked as ''no symptom'' and the right end marked as ''worst imaginable symptom), the verbal rating scale (VRS scores ranging 0–3, 0 = none, 1 = mild, 2 = moderate, 3 = severe), and the numerical rating scale[21] (NRS scores ranging 0–10, 0 = no pruritus, 10 = worst imaginable pruritus). In addition, dermatology life quality index[23] (DLQI, ranging 0–30) was also included.

The study was approved by the Health Science Research Ethics Board of Southeast University, the Ethical Committee of Peking University Shenzhen Hospital (No. 20160427), and conducted according to the Declaration of Helsinki principles. Written informed consent about all data for scientific research was obtained from all participants.

Simultaneous Quantitation of Five Steroids and Two Endocannabinoids in Plasma

The five steroids and two endocannabinoids in plasma were simultaneously detected by a novel analysis method using liquid chromatography tandem mass spectrometry (LC–MS/MS). The present analysis method showed good performances in linearity and limits of detection and quantification (*LOD* and *LOQ*). Validations of the method (i.e., intra-day and inter-day precision, recovery, matrix effect and stability in different conditions) also meet the acceptance criteria according to the FDA and EMA guidelines.[54,55] Detailed descriptions of method development, validation are given in the Supplementary Materials.

Statistical Analysis

Descriptive statistics were used to explore the characteristics of the sample. The normal distribution of data was examined by one-sample Shapiro–Wilk test. The endogenous levels of hormones and endocannabinoids in plasma were expressed as range and $M \pm SD$ in tables and $M \pm SEM$ in figure throughout the text where M is mean and SD is standard deviation and SEM is standard error of mean. Covariance analysis with age and gender as covariates was performed for the comparisons between the PN patients and controls in the contents of endogenous steroids, endocannabinoids, and their ratios in plasma. All statistical analyses were performed using SPSS.20 software for windows. Linearly fitting lines were plotted with the aid of Origin 9.0 software.

Results

The Consistency of the Four Scales in the Evaluation of Pruritus Intensity

The demographic information of participants are shown in Table 1. The intensities of pruritus and pain are evaluated by the visual analog scale (VAS), the prurigo score (PRUNOSI), the numerical rating scale (NRS) and verbal rating scale (VRS). As listed in Table 2, all the coefficients of Pearson's correlations (r) between the four scales were more than 0.7 ($ps < 0.001$), especially for very high correlation between VAS and NRS ($r = 0.932$). The intraclass correlation coefficient (*ICC*) among VAS, VRS and NRS scales was 0.760 (0.600–0.847, $p < 0.001$) as shown in Table 3. These results indicated that the four scales were highly consistent in the assessment of pruritus severity.

Comparison in Levels of Plasma Steroids and Endocannabinoids and their Ratios Between the PN Patients and Controls

As shown in Fig. 1, the PN patients showed significantly lower plasma contents than the controls in plasma cortisol, cortisone, progesterone as well as AEA ($ps < 0.05$), and significantly higher 1-AG contents ($p < 0.001$). In addition, lower plasma levels of the ratios of cortisone to cortisol, as well as the ratios of testosterone, AEA and DHEA to 1-AG ($p < 0.001$, $p < 0.01$ and $ps < 0.05$), higher levels of the ratios of testosterone and 1-AG to cortisol, and the ratio of testosterone to AEA ($p < 0.05$, $p < 0.01$ and $p < 0.05$) were observed in PN patients. Age only influenced the contents of cortisol ($F_{1,\ 68} = 4.9$, $\eta_p^2 = 0.057$, $p < 0.047$) and cortisone ($F_{1,\ 68} = 9.648$, $\eta_p^2 = 0.124$, $p < 0.$), but it was not true for the other biomarkers ($ps > 0.2$). Gender had no influences on the levels of all the potential biomarkers ($ps > 0.066$), which argued against age and gender influence on our findings.

Correlations of PRUNOSI, VAS, NRS, VRS and DLQI Scores with the Contents of Plasma Steroids and Endocannabinoids and their Ratios Among PN Patients

Furthermore, among the candidate biomarkers showing the intergroup differences, the association of contents of these candidates with the scores of four PN's symptoms (i.e., PRUNOSI, VAS, NRS, VRS) and DLQI were conducted to screen the biomarkers to assess the intensities of pruritus and pain. As listed in Table 4, among the endogenous contents of five biomarkers showing the intergroup differences, the contents of plasma cortisol showed highly negative correlation with all the scores of four PN's symptoms (i.e., PRUNOSI, VAS, NRS, VRS) ($r = -0.834$ to -0.936, $ps < 0.001$) and DLQI ($r = -0.938$ $p < 0.001$). Cortisone contents showed moderately or highly negative correlation with the scores of the four PN's symptoms and DLQI scores ($r = -0.602$ to -0.398, $ps < 0.05$). AEA contents also mostly showed moderately or highly negative correlation with the scores of the five scales ($r = -0.521$ to -0.358, $ps < 0.05$). Progesterone contents showed moderately and marginally negative correlation with the VAS score ($r = -0.315$, $p = 0.052$), but had no significant correlations with the other four scales ($r = -0.271$ to -0.214, $ps > 0.05$).

Among the seven ratio of candidate biomarkers showing the intergroup differences, the ratio of cortisone to cortisol in plasma was moderately and positively correlated with the scores of the five scales ($r = 0.429–0.504$, $ps < 0.05$). The ratio of testosterone to cortisol was also moderately and positively correlated with the scores of the five scales ($r = 0.341–0.506$, $ps < 0.05$). The ratio of 1-AG to cortisol was moderately and positively correlated with the scores of PRUNOSI, VAS, NRS and DLQI ($r = 0.471–0.539$, $ps < 0.01$),

Table 1 Baseline Characteristics Between PN Group and Healthy Control.

Variable	PN ($n = 36$)	Controls ($n = 36$)	*Statistical values*
Age (year)	47.2 ± 9.3	46.8 ± 9.1	$t_{70} = 0.306$, $p > 0.05$
Gender (male/female)	19/17	19/17	–
BMI (kg/m^2)	22.1 ± 2.5	23.3 ± 1.6	$t_{70} = -0.412$, $p > 0.05$

Table 2 Pearson's Correlation Coefficients (r) Between Visual Analog Scale (VAS), Verbal Rating Scale (VRS), Numerical Rating Scale (NRS) and the Prurigo Score (PRUNOSI), and Dermatology Life Quality Index (DLQI) among the PN Patients ($n = 36$).

	VAS	VRS	NRS	PRUNOSI
VAS	–			
VRS	0.813***	–		
NRS	0.932***	0.836***	–	
PRUNOSI	0.824***	0.846***	0.770***	–
DLQI	0.965***	0.881***	0.931***	0.867***

Notes:
*$p < 0.05$.
**$p < 0.01$.
*** $p < 0.001$.

Table 3 The Intraclass Correlation Coefficient (*ICC*) between/among VAS, VRS, NRS and PRUNOSI Scales in the PN Patients ($n = 36$).

	VAS	VRS	NRS	0.760**
VAS	–			–
VRS	0.600***	–		–
NRS	0.751***	0.813	–	–
PRUNOSI	0.745***	0.754***	0.712***	–
0.786***	–	–	–	–

Notes: *ICC* (VAS, VRS and NRS) = 0.760; *ICC* (VAS, VRS NRS PRUNOSI) = [illegible].
*$p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4 Coefficients of Partial Correlations Between PN's Psychological Symptoms Including the Scores of the Pruritus Severity, Pain and DLQI and Contents of Plasma Steroids and Endocannabinoids and their Ratios among the PN Patients ($n = 36$).

Bio-index	PRUNOSI	VAS	NRS	VRS	DLQI
F	−.85	−.936***	−.857***	−.834***	−.938***
E	4**	−.602***	−.457**	−.398*	−.569***
P	−0.	−.315#	−0.214	−0.271	−0.216
AEA	−.35	−.521**	−.364*	−.412*	−.438*
1-AG	165	−0.166	−0.174	−0.122	−0.138
E/F	.438**	.429*	.504**	.493**	.458**
AEA/1-AG	0.181	0.200	0.237	0.044	0.193
T/F	.460**	.506**	.386*	.341*	.447**
1-AG/F	.471**	.539**	.444**	.318#	.480**
T/AEA	−.413*	−.407*	−.382*	−.354*	−.341*
T/1-AG	0.126	0.162	0.103	−0.122	0.152
DHEA/1-AG	−0.145	−0.124	−0.207	−0.285	−0.16

Notes:
# $p < 0.1$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
F, cortisol; E, cortisone; T, testosterone; P, progesterone; DHEA, dehydroepiandrosterone; AEA, *N*-arachidonoylethanolamine; 1-AG, 1-arachydonoyl glycerol. PRUNOSI, the prurigo score; VAS, visual analog scale; VRS, verbal rating scale; NRS, numerical rating scale; DLQI, dermatology life quality index. Very high correlation, $r > 0.7$; high correlation, $0.5 < r < 0.7$; moderate correlation, $0.3 < r < 0.5$; weak correlation, $r < 0.3$.

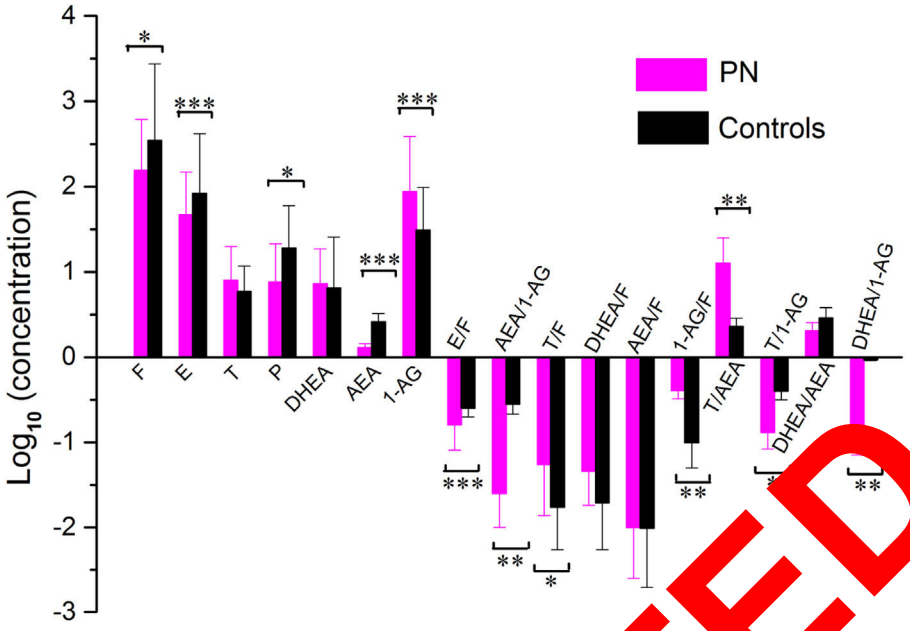

Figure 1 Comparison in candidates of plasma biomarkers between the PN pati[illegible] ($n = 36$) a[illegible]lthy controls ($n = 36$). Notes: F, cortisol; E, cortisone; T, testosterone; P, progesterone; DHEA, dehydroepian[illegible]oste[illegible]e; AEA, N[illegible]arachidonoylethanolamine; 1-AG, 1-arachydonoyl glycerol. The contents of all the seven compounds in plasma were re[illegible]ed in ng/mL. The levels of the 17 plasma biomarkers were presented as mean ± standard error of mean (SEM). T[illegible]or bar was [illegible]. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

but was moderately and marginally significantly positively correlated with the score of VRS ($r = 0.318$, $p = 0.058$). In contrast, the ratio of testosterone to AEA was modera[illegible] and negatively correlated with the scores of the five scal[illegible] ($r = -0.341$ to -0.413, $ps < 0.05$). The other rati[illegible] includ[illegible]ing AEA/1-AG, testosterone/1-AG and DHEA/[illegible]AG and no[illegible] correlations with the scores of the five scal[illegible] ($ps > 0.05$).

According to the screening criteria show[illegible] h[illegible] lations ($r > 0.5$) with the scores of all [illegible]ales, [illegible] cortisol, can be used as a biomarker to ac[illegible]ely assess[illegible]e PN's symptoms, especially for the pru[illegible]us se[illegible]ity. Additionally, In addition, cortisone, AEA, t[illegible] ratios of [illegible]isone to cortisol, testosterone to corti[illegible], 1-AG to cortisol and ratio of testosterone to AEA mig[illegible]be [illegible]d as potential biomarkers to assess the symptoms [illegible], due[illegible] showing moderate correlations ($0.3 <$ [illegible] with [illegible]of scales.

Discussions

In this study, twelve biomarkers from seventeen candidate biomarkers in plasma showed abnormal levels in the PN patients. To the best of our knowledge, it was the first investigation to verify the influence of PN on the three neuroendocrine systems including the HPA, HPG axes and ECS, as well as on the interactions within one endocrine system and between the three systems. Previous studies were confined only in the HPA axis showing abnormal activity in other skin diseases, such as chronic uticaria.[25,56] Moreover, seven biomarkers out of the twelve biomarkers showing the intergroup differences (i.e., cortisol, cortisone, AEA, the ratios of cortisone, testosterone and 1-AG to cortisol, and of testosterone to AEA ratio) were founded to be correlated with PN's symptoms, especially for the pruritus severity. The nine b[illegible]arkers [illegible]ght be potential biomarkers to accurately and obje[illegible]ssess the pruritus severity of PN. To the best of [illegible]ur knowledge, it was also the first attempt to screen sen[illegible]iomarkers for objectively assessing the PN's pruritus severity.

We found that plasma cortisol contents in PN patients were much lower than those in healthy controls (Fig. 1). Previous studies also found that there was a decreased cortisol level in chronic urticaria.[25,56] These results indicated that chronic skin diseases (e.g., prurigo nodularis and chronic urticarial) would cause hypocortisolism of the central HPA axis. Generally, when exposed to the acute stress, temporary activation of the central zone of the HPA axis occurs, resulting in the pulse secretion of cortisol and the increased cortisol levels.[27] In contrast, chronic stress may lead to the fatigue of the central HPA axis, following a hypocortisolism and a cortisol reduction.[57] Therefore, chronic skin diseases with pruritus and uncomfortable lesions (e.g., PN) would generate chronic stress, resulting in a hypocortisolism of the central HPA axis. In addition, the peripheral HPA axis in the skin might implicate in the PN's etiopathogenesis. The previous human studies have reported that the peripheral HPA axis may have regulatory mechanisms equivalent to the central HPA axis, with the with the epidermal, hair follicle keratinocytes, sebocytes, mast cells secreting corticotrophin-releasing hormone (CRH), and expressing CRH-R1 receptors in response to stress.[57,58] However, these pathomechanisms implicating the role of stress in the PN's etiopathogenesis need to be further validated in the future research.

The present study also found that PN patients showed lower plasma cortisone contents than healthy control (Fig. 1). This result also supported that the hypocortisolism in the HPA axis occurs in PN patients. This is because

inactive cortisone in plasma mainly results from the metabolism of active cortisol under the catalysis of 11β-hydroxysteroid dehydrogenase type 1 isozyme (11β-HSD1).[59] Previous studies have showed that cortisone levels were decreased in response to chronic stress.[60,61] These results gave an implication that cortisone is a reliable biomarker to assess the HPA activity same as cortisol. Naturally, the abnormalities in the HPA axis were likely to lead to the dysfunction of the HPG axis and ECS because the HPA and HPG axes and ECS regulate and interact with each other via secreting their respective terminal hormones or cannabinoids.[30,62] As a result, lower contents of plasma progesterone and AEA were observed in the current PN patients (Fig. 1), indicating that the hypocortisolism of the HPA axis is closely related to the impaired activity of the HPG axis and ECS among the PN patients. Conversely, the present PN patients showed higher the 1-AG contents than the controls (Fig. 1). Previous empirical studies have generally demonstrated that stress evokes bidirectional changes in the AEA and 1-AG levels, with chronic stress exposure decreasing AEA levels and increasing 1-AG levels.[63,64] Therefore, the findings on the decreased AEA levels and increased 1-AG levels in plasma were attributed to the stress from chronic pruritus and pain in the PN patients. In short, the current results revealed that PN patients might show the abnormality in the three endocrine systems, the HPA and HPG axes and ECS although there were no intergroup differences in testosterone and DHEA (Fig. 1).

Interestingly, the present study further found that PN patients showed lower levels than the controls in the ra of cortisone to cortisol, and the ratios of AEA, testostero and DHEA to 1-AG (Fig. 1). The ratio of cortisone to cor sol was used to assess not only the activity of 1 HSD1,[5] but also the connection within the HPA a [4,6] here-fore, it inferred on the present result th PN's tients might have the decreased activity of 1β- and th impaired interaction within the HPA xis in p llel with the above-mentioned hypocortisol der chro stress of PN's itching and pain. Previous studie d also demonstrated that elite athletes n long-term aining stress showed lower levels in the io of rtisol to cortisone, such as cyclists.[66] These result dicated that the ratio of cortisol to cortisone is a useful indic r of chronic stress. Similarly, because of A -AG reflects the interaction within EC and e ratios f testosterone and DHEA to 1-AG reflect between the HPG axis and ECS,[67] it referred PN's patients might have the weakened interactions wit ECS and between the HPG axis and ECS. However, the interaction the HPG axis and ECS through the testosterone-AEA pathway might be enhanced in PN's patients showing higher levels of the ratio of testosterone to AEA than the controls (Fig. 1) due to their decreased AEA contents and nearly unchanged testosterone contents. The distinct mechanism between AEA and 1-AG might be attributed to the distinctly bidirectional changes in the AEA and 1-AG levels under the exposure of chronic stress[68,69] as mentioned above. With regard to the interactions of the HPA axis with the HPG axis and ECS, the PN patients showed higher levels in the ratios of testosterone and 1-AG to cortisol than the controls (Fig. 1) because the decreased contents of cortisol and AEA and the increase in 1-AG contents, and no significant difference in testosterone contents between the two groups were observed in the PN group (Fig. 1). It could be possibly inferred that the interactions of the HPA axis with the HPG axis and ECS were enhanced in PN's patients because they were used to assess the interactions of the HPA axis with the HPG axis and ECS, respectively. In the previous, the increased ratio of testosterone to cortisol was observed in individuals with higher psychopathy scores, indicating that they showed the augmented interaction between the HPA and HPG axes.[70]

Finally, the present study found that plasma contents of cortisol and cortisone showed moderately and over negative correlations with the PN's symptoms, especially for PRUNOSI and VAS (Table 4). The previous study had demonstrated that cortisol and cortisone were negatively correlated with the disease severity of chronic urticarial.[60] Thus, cortisol and cortisone might to some e lect the PN's pruritus severity except that ar omarker f the PN-induced chronic stress and the activ n degre of the HPA axis. Additionally, AEA cont s had der ly and over negative correlations wi the P's pr s severity (Table 4). Therefore, plasm rtis , cortisone, and AEA showing the intergroup ffer es and oderately and over correlated with t PN's sym can be sensitive and reliable biomarke fo valuating e pruritus severity of PN. Furthermore, this s y found that the ratios of cortisone to co nd of testo rone to AEA, of testosterone and 1-AG t cortisol showing the intergroup differences were mode tely and er correlated with the PN's pruritus severity (T 4). It o implied that the four ratios also can be sensitive tiable biomarkers for assessing the PN's pruritus erity. In the previous, the ratio of cortisol to cortisone en proven to be a useful bio-indicator for monitoring the overreaching state and performance of elite swimmers with long-term training.[66,71]

This study has three limitations. Firstly, the small sample may limit the power of this study in examining the intergroup differences and the correlations between PN's psychological symptoms and biomarkers among the PN patients. Secondly, our study used a cross-sectional design, which excludes conclusions about the causality and directionality of the connotations. In order to explore correlations between the physiological and psychological responses elicited by skin disease, a longitudinal design with larger-scale samples will be helpful for better understanding of chronic stress which plays a role in pathogenesis of PN in the future. Finally, the activity of the HPA is very complex and fluctuating during the day. Certainly, one single plasma spot of cortisol and cortisone is insufficient to picture the axis's activity in this study. Thus, at least a 24-hour urine sample and multiple circadian sampling are needed to have a clear idea of the activity of the HPA in the future.

Conclusions

The PN patients showed abnormal levels in twelve biomarkers from seventeen candidate biomarkers in plasma (i.e., cortisol, cortisone, progesterone, AEA, 1-AG, the ratios of cortisone, testosterone and 1-AG to cortisol, the ratios of testosterone, DHEA and AEA to 1-AG, the ratio of testosterone to AEA). These results indicated that PN patients could show the abnormality in the three endocrine systems

including the HPA and HPG axes and ECS, as well as in the interaction within one endocrine system and between the three systems. Furthermore, the PN's symptoms, especially for the PN's pruritus severity were moderately and over correlated with seven biomarkers showing the intergroup differences (i.e., cortisol, cortisone, AEA, the ratios of cortisone, testosterone and 1-AG to cortisol, and of testosterone to AEA). The seven biomarkers would be sensitive and reliable biomarkers for assessing the pruritus severity of PN because they met the screening criteria that the biomarkers show intergroup differences and showed moderate or over correlation with the pruritus severity of PN. This study could provide valuable insights into the physiological mechanism of psychological responses involved in the pathogenesis of PN.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Zeidler C, Tsianakas A, Pereira M, St er H, povitch G, Stander S. Chronic prurigo of nodula : a review ta Derm Venereol. 2018;98:173–9.
2. Schneider G, Hockmann J, Stu f A. Psych matic aspects of prurigo nodularis. Hautarzt 4;65:704–8.
3. Jorgensen KM, Egeberg A slason l, Skov L, Thyssen JP. Anxiety, depression and suic in ients with prurigo nodularis. J Eur Acad Dermatol Venere 017;31 06–7.
4. Brenaut E, Halv , Dal , Lien L, Misery L. The self-assessed cholo al comorbidities of prurigo in european patient mul tudy in 13 countries. J Eur Acad Dermatol Vener 018;33:157–62.
5. Tseng HC, Tsai WC, C, Lee CH, Chen CC, Lee YT, et al. Prurigo nodularis is associate ith depression and anxiety in chronic hemodialysis patients. Acta Nephrol. 2015;29:94–101.
6. Brenaut E, Halvorsen JA, Dalgard FJ, Lien L, Balieva F, Sampogna F, et al. The self-assessed psychological comorbidities of prurigo in european patients: a multicentre study in 13 countries. J Eur Acad Dermatol Venereol. 2019;33:157–62.
7. Schneider G, Hockmann J, Stander S, Luger TA, Heuft G. Psychological factors in prurigo nodularis in comparison with psoriasis vulgaris: results of a case–control study. Br J Dermatol. 2006;154:61–6.
8. Schneider G, Hockmann J, Stander S, Luger TA, Heuft G. Psychological factors in prurigo nodularis in comparison with psoriasis vulgaris: results of a case–control study. Br J Dermatol. 2006;1541:61–6.
9. Mazzetti M, Mozzetta A, Soavi GC, Andreoli E, Bonda PGF, Puddu P, et al. Psoriasis, stress and psychiatry – psychodynamic characteristics of stressors. Acta Dermato-Venereol. 1994;74:62–4.
10. Alabadie MS, Kent GG, Gawkrodger DJ. The relationship between stress and the onset and exacerbation of psoriasis and other skin conditions. Br J Dermatol. 1994;130:199–203.
11. Mollanazar NK, Smith PK, Yosipovitch G. Mediators of chronic pruritus in atopic dermatitis: getting the itch out? Clin Rev Allergy Immunol. 2016;51:263–92.
12. Schwendinger-Schreck J, Wilson SR, Bautista DM. Interactions between keratinocytes and somatosensory neurons in itch. Handb Exp Pharmacol. 2015;226:177–90.
13. Dazzi C, Erma D, Piccinno R, Veraldi S, Caccialanza M. Psychological factors involved in prurigo nodularis: a pilot study. J Dermatolog Treat. 2011;22:211–4.
14. Schut C, Mollanazar NK, Kupfer J, Gieler U, Yosipovitch G. Psychological interventions in the treatment of chronic itch. Acta Derm Venereol. 2016;96:157–6
15. Florence J, Dalgard U, To -Arago L, Lien L, Poot F, Gregor BE, et al. The psy ogical bu n of skin diseases: a cross-sectional mul cente tudy a ng dermatological out-patients in 13 opean co ie Investig Dermatol. 2015;135:984–91
16. Zigmond AS, Sna RP me hospital anxiety and depression scale. Acta chiat and. 19 ;67:361–70.
17. Oh CC, Li Lee W, Te psychosocial factors associated with p g dularis in dogenous eczema. Indian J Dermatol. 2015;60:5
18. NQ, Blome Fritz F, Gerss J, Reich A, Ebata T, et al. Assessment of pruritus intensity: prospective study on validity and reliab ty of the visual analogue scale, numerical rating scale and bal rating scale in 471 patients with chronic pruritus. Acta rm Venereol. 2012;92:502–7.
19. Lis Severity scoring of atopic dermatitis: the scorad index. Consensus report of the european task force on atopic dermatitis. Dermatology. 1993;186:23–31.
20. Siepmann D, Lotts T, Blome C, Braeutigam M, Phan PQ, Augustin MET-AL>. Evaluation of the antipruritic effects of topical pimecrolimus in non-atopic prurigo nodularis: results of a randomized, hydrocortisone-controlled, double-blind phase ii trial. Dermatology. 2013;227:353–60.
21. Williamson A, Hoggart B. Pain: a review of three commonly used pain rating scales. J Clin Nurs. 2005;14:798–804.
22. Siepmann D, Lotts T, Blome C, Braeutigam M, Phan NQ, Butterfass-Bahloul T, et al. Evaluation of the antipruritic effects of topical pimecrolimus in non-atopic prurigo nodularis: results of a randomized, hydrocortisone-controlled, double-blind phase ii trial. Dermatology. 2013;227:353–60.
23. Finlay AY, Khan GK. Dermatology life quality index (dlqi) – a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19:210–6.
24. Sonia Louise D, Robin B. Androgen physiology. Semin Reprod Med. 2006;24:71–7.
25. Varghese R, Rajappa M, Chandrashekar L, Kattimani S, Archana M, Munisamy M, et al. Association among stress, hypocortisolism, systemic inflammation, and disease severity in chronic urticaria. Ann Allergy Asthma Immunol. 2016;116:344–8.
26. Brzoza Z, Kasperska-Zajac A, Badura-Brzoza K, Matysiakiewicz J, Hese RT, Rogala B. Decline in dehydroepiandrosterone sulfate observed in chronic urticaria is associated with psychological distress. Psychos Med. 2008;70:723–8.
27. Varghese R, Rajappa M, Chandrashekar L, Kattimani S, Archana M, Munisamy M, et al. Association among stress, hypocortisolism, systemic inflammation, and disease severity in chronic urticaria [Article]. Ann Allergy Asthma Immunol. 2016;116:344–8, e1.
28. Mlynek A, Maurer M, Zalewska A. Update on chronic urticaria: focusing on mechanisms [Review]. Curr Opin Allergy Clin Immunol. 2008;8:433–7.

29. Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34:163–71.
30. Gorzalka BB, Dang SS, Minireview:. Endocannabinoids and gonadal hormones: bidirectional interactions in physiology and behavior. Endocrinology. 2012;153:1016–24.
31. Handa RJ, Weiser MJ. Gonadal steroid hormones and the hypothalamo–pituitary–adrenal axis. Front Neuroendocrinol. 2014;35:197–220.
32. Handa RJ, Weiser MJ. Gonadal steroid hormones and the hypothalamo–pituitary–adrenal axis. Front Neuroendocrinol. 2013;35:197–220.
33. Vincenzo M, Filippo D. Endocannabinoid system, stress and hpa axis. Eur J Pharmacol. 2018;834:230–9.
34. Roubinov DS, Boyce WT, Lee MR, Bush NR. Evidence for discrete profiles of children's physiological activity across three neurobiological system and their transitions over time. Dev Sci. 2020:e12989.
35. Oyola MG, Handa RJ. Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: sex differences in regulation of stress responsivity. Stress. 2017;20:476–94.
36. Bae YJ, Reinelt J, Netto J, Uhlig M, Willenberg A, Ceglarek U, et al. Salivary cortisone, as a biomarker for psychosocial stress, is associated with state anxiety and heart rate. Psychoneuroendocrinology. 2019;101:35–41.
37. Kudielka BM, Hellhammer DH, Kirschbaum C. The impact of age, gender, and sex steroids on psychological and endocrine responses to psychosocial stress and dex-crf in healthy elderlies and young controls: findings in three placebo controlled double blind studies. Psychoneuroendocrinology. 2000;25:S54.
38. Mezzullo M, Fanelli F, Di Dalmazi G, Fazzini A, Ibarra-Gasparini D, Mastroroberto M, et al. Salivary cortisol and cortisone responses to short-term psychological stress challenge in adolescent and young women with different hyperandrogenic states. Psychoneuroendocrinology. 2018;91:31–40.
39. Hill MN, Bierer LM, Makotkine I, Golier JA, Galea S, Ewen BS, et al. Reductions in circulating endocannabinoid levels in individuals with post-traumatic stress disorder following exposure to the world trade center attacks. Psychoneuroendocrinology. 2013;38:2952–61.
40. Coccaro EF, Hill MN, Robinson D, Lee RJ. Circulating endocannabinoids and affect regulation in human subjects. Psychoneuroendocrinology. 2018;92:66–71.
41. Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology. 2012;37:589–601.
42. Iwasa T, Matsuzaki T, Kinoshita M, Yano K, Tungalagsuvd A, Munkhzaya M, Kuwahara A, Yamamoto Y, Irahara M. The roles of kisspeptin and gonadotropin inhibitory hormone in stress-induced reproductive dysfunctions. Endocr J. 2018;65:133–40.
43. Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic–pituitary–gonadal axis: peripheral and central mechanisms. Biol Reprod. 1991;45:523–32.
44. Micale V, Drago F. Endocannabinoid system, stress and hpa axis. Eur J Pharmacol. 2018;834:230–9.
45. Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TTY, Gray JM, et al. Endogenous cannabinoid signaling is essential for stress adaptation. Proc Natl Acad Sci U S A. 2010;10720:9406–11.
46. Hill MN, Tasker JG. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic–pituitary–adrenal axis. Neuroscience. 2012;204:5–16.
47. Chailurkit LO, Aekplakorn W, Paiyabhroma N, Ongphiphadhanakul B. Global 11 beta-hydroxysteroid dehydrogenase activity assessed by the circulating cortisol to cortisone ratio is associated with features of metabolic syndrome. Metab Syndr Relat Disord. 2020.
48. Stephens MA, Mahon PB, McCaul ME, Wand GS. Hypothalamic–pituitary–adrenal axis response to acute psychosocial stress: effects of biological sex and circulating sex hormones. Psychoneuroendocrinology. 2016;66:47–55.
49. Asadikaram G, Khaleghi E, Sayadi A, Foulady S, Ghasemi MS, Abolhassani M, et al. Assessment of hormonal alterations in major depressive disorder: a clinical study. Psych J. 2019;8:423–30.
50. Markopoulou K, Papadopoulos A, Juruena MF, Poon L, Pariante CM, Cleare AJ. The ratio of cortisol/dhea in treatment resistant depression. Psychoneuroendocrinology. 2009;34:19–26.
51. Palkovits M, Harvey-White J, Liu J, Kovacs ZS, Bobest M, Lovas G, et al. Regional distribution and effects of postmortal delay on endocannabinoid content of the human brain. Neuroscience. 2008;152:1032–9.
52. Wu Y, Wu Y, Deng J, Chu L, Yang H, Wang W, et al. Screening and identification of salivary biomarkers assessing the effects of exogenous testosterone administration on hpg and hpa axes and ecs. Steroids. 2020;158:10[illegible].
53. Aitken RC. Measurement of feelings using visual analogue scales. Proc R Soc Med. 1969;62:989–93.
54. Kux L. Draft guidance for industry and food and drug administration staff; accepting electronic filing review for premarket approval applications; availability. Federal Register. 2012.
55. Smith G, European Medicines Agency (EMA), guideline on bioanalytical method validation, 21 July 2011. Bioanalysis. 2012;4:865–8.
56. Uzuner D, Akkurt I, Ucmak F, Toprak G, Acar G, Arica M. Investigation of dermatology life quality index and serum prolactin and serum dehydroepiandrosterone sulphate levels in patients with chronic urticaria. Asian Pac J Allergy Immunol. 2014;32:[illegible]–9.
57. Ruan Y, Du G, Dan L, Yuan TF. A possible change process of inflammatory cytokines in the prolonged chronic stress and its ultimate implications for health. Sci World J. 2014:1–8, 2014.
58. Kim JE, Cho BK, Cho DH, Park HJ. Expression of hypothalamic–pituitary–adrenal axis in common skin diseases: evidence of its association with stress-related disease activity. Acta Derm Venereol. 2013;93:387–93.
59. Lopez Bernal A, Anderson ABM, Turnbull AC. Cortisol: cortisone interconversion by human decidua in relation to parturition: effect of tissue manipulation on 11β-hydroxysteroid dehydrogenase activity. J Endocrinol. 1982;93:141–9.
60. Lee JH, Bae YJ, Lee SH, Kim SC, Lee HY, Ban GY, et al. Adaptation and validation of the korean version of the urticaria control test and its correlation with salivary cortisone. Allergy Asthma Immunol Res. 2019;11:55–67.
61. Blair J, Adaway J, Keevil B, Ross R. Salivary cortisol and cortisone in the clinical setting. Curr Opin Endocrinol Diabetes Obes. 2017;24:161–8.
62. Chu LX, Liu WH, Deng J, Wu Y, Yang HR, Wang W, et al. Age-related changes in endogenous glucocorticoids, gonadal steroids, endocannabinoids and their ratios in plasma and hair from the male c57bl/6 mice. Gen Comp Endocrinol. 2021:301.
63. Kim JE, Cho BK, Cho DH, Park HJ. Expression of hypothalamic–pituitary–adrenal axis in common skin diseases: evidence of its association with stress-related disease activity. Acta Dermato-Venereol. 2013;93:387–93.
64. Chu LX, Li N, Deng J, Wu Y, Yang HR, Wang W, et al. Lc-apci(+)-ms/ms method for the analysis of ten hormones and two endocannabinoids in plasma and hair from the mice with different gut microbiota. J Pharm Biomed Anal. 2020;185:12.
65. Chu L, Huang Y, Xu Y, Wang L-K, Lu Q. An lc-apci+-ms/ms-based method for determining the concentration of neurosteroids in the brain of male mice with different gut microbiota. J Neurosci Methods. 2021;360:109268.
66. Gatti R, Cappellin E, Zecchin B, Antonelli G, Spinella P, Mantero F, et al. Urinary high performance reverse phase

chromatography cortisol and cortisone analyses before and at the end of a race in elite cyclists. J Chromatogr B. 2005;824:51–6.
67. Kim J, Carlson ME, Watkins BA. Docosahexaenoyl ethanolamide improves glucose uptake and alters endocannabinoid system gene expression in proliferating and differentiating c2c12 myoblasts. Front Physiol. 2014;5:100.
68. Morena M, Patel S, Bains JS, Hill MN. Neurobiological interactions between stress and the endocannabinoid system. Neuropsychopharmacology. 2016;41:80–102.
69. Yasmin F, Colangeli R, Morena M, Filipski S, van der Stelt M, Pittman QJ, et al. Stress-induced modulation of endocannabinoid signaling leads to delayed strengthening of synaptic connectivity in the amygdala. Proc Natl Acad Sci U S A. 2020;1171:650–5.
70. Glenn AL, Raine A, Schug RA, Gao Y, Granger DA. Increased testosterone-to-cortisol ratio in psychopathy. J Abnorm Psychol. 2011;1202:389–99.
71. Atlaoui D, Duclos M, Gouarne C, Lacoste L, Barale F, Chatard JC. The 24-h urinary cortisol/cortisone ratio for monitoring training in elite swimmers. Med Sci Sports Exerc. 2004;36:218–24.