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ORIGINAL RESEARCH

Relationship of Substance P with the Degree of Atopic Dermatitis Severity

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Introduction: Atopic dermatitis (AD) is a chronic inflammatory skin disease that starts during childhood with a varied course. Itching or incessant itching in severe cases, sleep disturbance, and infection-prone skin are the typical symptoms of this disease. Substance P is postulated to have an important role in AD. Increasing levels of substance P in AD induce the release of IFN-γ, IL-4, tumor necrosis factor-α (TNF-α), and IL-10 from peripheral blood mononuclear leukocytes, inducing an itching response.

Methods: This study is a cross-sectional study that aims to analyze the relationship between serum substance P levels and AD severity in children using Score of AD (SCORAD), and to determine the mean serum substance P levels and severity of AD in patients with AD. This study also aims to find out the correlation of substance P levels with the SCORAD values.

Results: Forty-six children (29 males; 17 females) with a mean age of 10.35 years (standard deviation (SD) = 4.01) were diagnosed with AD. The SCORAD index assessment was conducted to analyze AD (mean value, 23.15; SD = 9.42), and mild AD obtained the highest degree. The level of substance P was also examined (mean value, 300.88; SD = 127.55).

Discussion: This study did not find a significant relationship between substance P levels and AD severity (p-value = 0.880), and there was no significant correlation between substance P levels and SCORAD values (p-value = 0.233; r = −0.179). The limitations of this study include a small number of cases, no control group, and we only found two cases of severe AD. To generalize the results, further studies with wide range population and AD severity might be done in the future.

Keywords: atopic dermatitis, SCORAD, substance P, pruritus

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease that begins during childhood with a varied course. The typical symptoms of this disease are itching, sleep disturbance, skin excoriation, and infection-prone skin.[1]

AD is a major health problem worldwide with a prevalence of 15–30% in children and 2–10% in adults. The International Study of Asthma and Allergies in Childhood (ISSAC) found that the morbidity rate reached 20% in Asian countries such as South Korea, Taiwan, and Japan. In developing countries, AD is estimated to be prevalent in 10–20% of children, 60% of which persists in adulthood.[2] In Indonesia, according to the Indonesian Pediatric Dermatology Study Group (KSDAI), the prevalence rate of AD cases reaches around 23.6%, where AD ranked first in the top 10 skin diseases in children.[3]

The pathogenesis of AD remains not fully understood. It is believed that there is a complex mechanism by which the interaction between genetics, skin barrier

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dysfunction, and immunology occurs.[4] Skin neuropeptides, particularly substance P, contribute to the pathogenesis of various skin diseases, one of which is AD. Substance P promotes the production of nerve growth factors from keratinocytes and the release of histamine, leukotriene, or tumor necrosis factor (TNF) from mast cells, causing the growth of sensory nerve fibers and augmentation of skin inflammation. Therefore, substance P is currently considered as one of the key pruritogenic factors in AD.[5]

The major substance P receptor on skin mast cells is Mas-related G protein-coupled receptor-X2 (MRGPRX2).[6] Substance P is synthesized and released from mast cells, monocytes, and eosinophils in the skin. Substance P, along with IL-17A, appears to be significantly elevated in the plasma of patients with AD.[7] The components of the skin microbiome can stimulate host defense peptide production in keratinocytes, of which β-defensin and cathelicidin induce MC degranulation via MRGPRX2 and also the changes in MRGPRX2 itself or elements upstream or downstream thereof (transcription factors, signaling components) may predispose to AD development.[6] Recent studies have also shown that substance P also induces the expression of endothelial cell attachment molecules such as P-selectin to a limited extent. The expression of high levels of P-selectin in the dermal vessels in skin lesions has been observed in urticaria and AD.[8]

This study aims to analyze the relationship between serum substance P levels and the severity of AD in children, determine the mean serum substance P levels in patients with AD, and determine the degree of severity of AD in patients with AD, as well as determine the relationship between substance P levels and the degree of AD severity and the correlation of substance P levels with the Score of Atopic Dermatitis (SCORAD) values.

Methods

This research is a cross-sectional study and was conducted at the Polyclinic of Dermatology and Venereology, Sumatera Utara University Hospital. Blood samples were examined at Prodia Laboratory in Medan. This study is done from February to November 2020, with the approval from Health Research Ethical Committee Faculty of Medicine Universitas Sumatera Utara Number 92/KEP/USU/2020.

The subjects of this study were female and male patients with AD diagnosed based on Hanifin and Rajka's criteria and parent or legal guardian provided informed consent for those who were willing to take part in the study. Subjects will be excluded if their parents/guardians cannot answer the questions given and are suspected of having skin diseases or other systemic diseases. This study was conducted in accordance with the Declaration of Helsinki. Forty-six children with AD were identified and then evaluated for the degree of severity using the SCORAD index.

Blood samples were then taken to check substance P levels using the substance P ELISA kit from the R&D Systems catalogs. Then, reagents, working standards, and samples were prepared. The excess microplate strip from the plate frame was removed, then returned to the foil pouch containing the desiccant pack, and reinstalled. Diluent RD5-45 Calibrator (100 µL) was then added to the nonspecific binding (NSB) well. This is followed by the addition of Diluent Calibrator RD5-45 (50 µL) to the standard zero well (B0); then, 50 µL of standard, control, or sample were added to the remaining wells. Plate layouts were provided to record the standards and samples tested.

This procedure was continued by adding 50 µL of primary antibody solution to each well (excluding NSB well). All wells, except the NSB well, turned blue. Then, 50 µL of substance P conjugate was added to each well. All wells, except NSB well, now turned purple. Wells were then covered with the provided adhesive strip. Samples were incubated for 3 hours at room temperature on a horizontal orbital microplate shaker (orbit, 0.12") adjusted at 500 ± 50 rpm.

Each well was then aspirated and washed four times. Each well was washed by filling each well with a wash buffer (400 µL) using a spray bottle, dispenser manifold, or auto washer. Fluid discharge at every step is essential for good performance. After the fourth wash, the remaining buffer was removed by vacuuming or pouring. The plate was then turned over and cleaned using a clean paper towel. Then, 200 µL of substrate solution was added to each well. Samples were incubated for 30 minutes at room temperature on a table and protected from mild. Stop solution (50 µL) was then added to each well.

The optical density of each well was determined within 30 minutes using a microplate reader set at 450 nm. If wavelength correction is available, it should be set to 540 or 570 nm. If wavelength correction is unavailable, the reading should be reduced at 540 or 570 nm from 450 nm. This reduction will correct for optical imperfections in the plate. Readings directly made at 450 nm without correction may be higher and less accurate. Then, the results will be presented in units of pg/mL.

Data on the distribution of sex and the degree of severity of patients with AD were presented in the form of a descriptive table. Data on the distribution of patients with AD were reported as means (median ± standard deviation (SD)). The results of the SCORAD index assessment and the measurement of substance P levels were reported as mean values (median ± SD). The relationship of substance P levels with the degree of AD severity was determined using the Kruskal–Wallis test, and the relationship between substance P levels and SCORAD was tested using the Spearman correlation.

Result

A total of 46 children with AD were enrolled in this study. They visited the Polyclinic of Dermatology and Venereology Universitas Sumatera Utara Hospital, Medan, from February 2020 to November 2020. The inclusion and exclusion criteria were met. There were 29 male subjects (63%) and 17 female subjects (37%) (Table 1).

The mean age of subjects with AD who were enrolled in the study was 10.35 years (SD = 4.01), with the youngest being 1 year old and the oldest being 17 years old (Table 2). Table 3 presents the results of the SCORAD assessment of all research subjects. The mean SCORAD was 23.15 (SD = 9.42), with the lowest SCORAD being 10.8 and the highest being 59.6.

The results of the SCORAD index categorization indicated that majority of study subjects experienced AD with mild or mild severity (n = 33; 71.7%), followed by moderate (n = 11; 23.9%) and severe (n = 2; 4.3%) (Table 4). Table 5 shows the results of the examination of substance P levels, and the mean value of substance P levels from the 46 patients with AD was 300.88 (SD = 127.55), with the lowest level being 172.4 pg/mL and the highest being 764.4 pg/mL.

The relationship of substance P levels with the degree of AD severity (Figure 1) was analyzed with Kruskal–Wallis test. The results showed that there was no significant relationship between the level of substance P and the severity of AD (SCORAD category) (p = 0.880) (Table 6). Although it did not show a significant relationship, it was seen that subjects with severe AD had the lowest median value of 265.1 (range, 262.8–267.4); it was slightly higher at moderate degree of severity, with a median of 270.21 (range, 177.6–372.7); and mild or mild degree of severity

Table 1 The Frequency Distribution of Gender of the Research Subjects

Gender	Frequency	%
Male Female	29 17	63 37
Total	46	100

Table 2 Age Distribution of the Research Subjects

Age	n = 46
Average	10.35
Standard Deviation (SD)	4.01
Median	11
Min–Max	1–17

Table 3 Distribution of SCORAD of the Research Subjects

SCORAD	n = 46
Average	23.15
Standard Deviation (SD)	9.42
Median	22
Min–Max	10.8–59.6

Table 4 Severity of Atopic Dermatitis (SCORAD Category)

Atopic Dermatitis Severity	Frequency	%
Mild	33	77.1
Moderate	11	22.9
Severe	2	4.3
Total	46	100

Table 5 Distribution of Substance P Levels in the Subjects

Level of Substance P	n = 46
Average	300.88
Standard Deviation	127.55
(SD)	270.50
Median	172.4–764.4

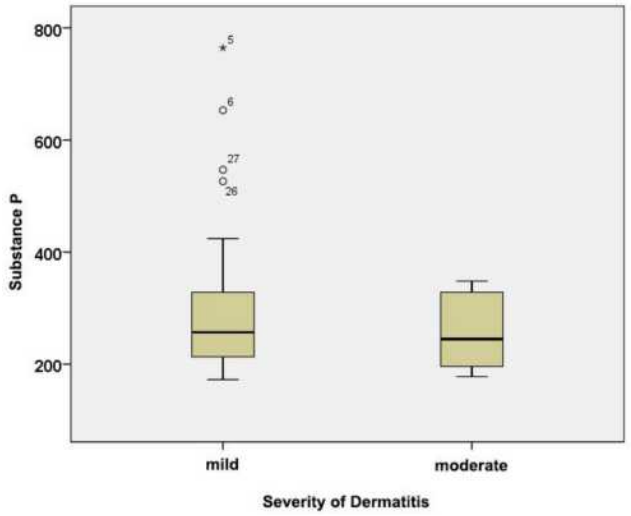

Figure 1 Boxplot Graph of the Relationship Between Substance P Levels and Degree of AD Severity.

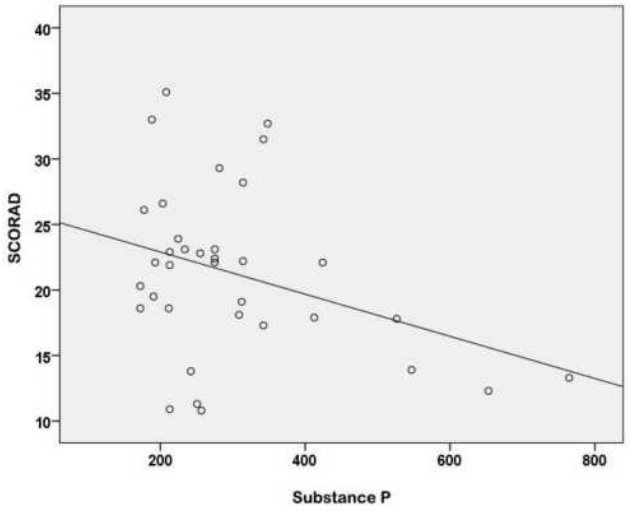

Figure 2 Scatterplot Graph of the Correlation Between Substance P Levels and SCORAD Value.

had the highest median value, which was 273.6 (range, 172.4–764.4).

The relationship between substance P levels and SCORAD (Figure 2) was analyzed using the Spearman correlation. There was no significant correlation between the level of substance P and the SCORAD value ($p = 0.233$; $r = -0.179$), as shown in Table 7.

Discussion

AD is a major health problem worldwide with prevalence rates of 15–30% in children and 2–10% in adults. The ISSAC found that the morbidity rate reached 20% in Asian countries such as South Korea, Taiwan, and Japan. The AD ratio in women is higher than in men (1.5:1), and AD often starts early in the growth period. In children, 45% of AD cases first appeared at the age of 6 months, 60% appeared at the first year of age, and 8% first appeared before the age of 5 years.[9,10] In our study, it was found that there was more frequency in males, with an average age of 10 years.

AD severity was assessed using the SCORAD. The SCORAD index was developed by the European Task Force on AD (ETFAD) in 1993 and is one of the most frequently used measures to assess AD severity. The SCORAD assesses the extent of skin lesions, intensity of the morphology of the lesions, and subjective complaints.[11,12] In our study, it was found that mild AD is the most common form of AD in the study subjects.

Several studies support the important role of neuropeptides in the pathophysiology of pruritus in various skin diseases.[8] MRGPRX2 is the major substance P receptor on skin mast cells, causing an increased inflammatory response and predisposing AD development.[6] Substance P has been postulated to have an important role in AD.[5] Plasma levels of the neuropeptide substance P are elevated in patients with AD and remain elevated even after remission. Increased levels of substance P in AD induce the release of IFN-γ, IL-4, TNF-α, and IL-10 from peripheral blood mononuclear leukocytes.[13] However, our study found that

Table 6 Relationship Between Substance P Levels and Degree of AD Severity

Atopic Dermatitis Severity	n	Substance P, Median (Range)	*p*
Mild	33	273.6 (172.4–764.4)	0.880
Moderate	11	270.21 (177.6–372.7)	
Severe	2	265.1 (262.8–267.4)	

Table 7 Relationship Between Substance P Levels and SCORAD Value

	SCORAD	
	P	r
Substance P	0.233	−0.179

there was no relationship between the level of substance P and the severity of AD.

The limitations of this study include a small number of cases, no control group, and we only found two cases of severe AD. To generalize the results, further studies with wide range population and AD severity might be done in the future.

Conclusion

Most subjects in AD were male, with a mean age of 10.09 years, the youngest being 1 year old and the oldest being 17 years old. Majority of the subjects had mild AD. In addition, there was no significant relationship between the level of substance P and the severity of AD, and no significant correlation was found between the level of substance P and the SCORAD value.

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Disclosure

The authors report no conflicts of interest in this work.

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