

# Correlation between grooming and scratching behavior in BALB/c mice related to itch sensation caused by house dust mite allergen

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## Abstract

**Objective** Self-grooming can be induced by stressful situations. External stimuli such as house dust mites (HDM) allergen can cause acute itch sensations that lead to scratching behavior. This pilot study compared grooming and scratching behavior between mice that exposed to allergen and placebo as well as the correlation between the two behaviors.

**Methods** This experimental pilot study involved six to eight weeks old male BALB/c mice, divided randomly into 2 groups, 8 mice each. The first group underwent the treatment of patch and spray with *Dermatophagoides pteronyssinus* allergen extract, while the second group was given placebo. The assessment for grooming and scratching was done on day 8. Grooming behavior assessed in this study consisted of total grooming chain with cephalocaudal pattern, grooming with non-cephalocaudal pattern, interrupted grooming bouts, body grooming, and total grooming duration.

**Results** The amount of grooming activity (number of bouts and time spent grooming) and number of non-chain (non-cephalocaudal pattern) grooming as well as the scratching bouts were higher in HDM allergen group compared to placebo group. The agreement between two researchers for grooming and scratching evaluation was assessed and showed substantial to almost perfect agreement with  $\kappa$  ranged between 0.535-0.911 and  $p < 0.001$ . The difference between two groups was significant for the scratching bouts ( $p=0.017$ ), non-chain patterned grooming ( $p=0.022$ ), body grooming ( $p=0.014$ ) and grooming duration ( $p=0.008$ ). The correlation between scratching bouts and total grooming duration, scratching bouts and non-chain patterned grooming, as well as scratching bouts and body grooming had high positive correlation.

**Conclusion** House dust mite allergen increased grooming and scratching behavior in mice compared to placebo. There were positive correlations between scratching behavior and non-chain patterned grooming, body grooming and duration of grooming, which could indicate the anxiety or stressful situation caused by itchiness from HDM allergen.

## Key words

Grooming; Scratching behavior; BALB/c; Atopic dermatitis; House dust mites; Tropical disease.

## Introduction

Animal self-grooming is a natural behavior associated with hygiene care as well as other

physiologically significant activities including thermoregulation, social communication, and de-arousal. It has a structured, sequential steps with typical cephalocaudal movement and is one

of the most commonly seen behaviors in mouse.<sup>1</sup> The assessment of self-grooming in mice or rodents were mostly related to stress, anxiety, or de-arousal.<sup>1-3</sup> While self-grooming in mouse is not an exact model of any human disease, its excessive and maladaptive form, along with its repetitive and stereotyped elements, makes it particularly helpful for modeling human compulsions exacerbated by stress. Self-grooming in humans can become pathological under specific circumstances, such as during stressful situations or in the presence of certain neuropsychiatric diseases.<sup>1,3</sup>

Self-grooming in mice and rats involves a sequence of individual movements, including highly stereotyped patterns, and demonstrates a high level of behavioral complexity and organization. There are several brain regions involved in self-grooming according to the studies, such as the basal ganglia (especially the striatum), neocortex, cerebellum, amygdala, hypothalamus, and brainstem. The amygdala of limbic system controls the modulation of emotional states including fear, anxiety, and desire. The state of anxiety or panic will increase self-grooming while depression will decrease the grooming activity. Furthermore, anxiety is proposed to be associated with disrupted grooming pattern.<sup>1</sup> Self-grooming can be induced by exposing the mouse into stressful situations which is the most common method used in studies about mouse self-grooming. Artificial-induced grooming is done by making the mouse in direct contact with substance, such

as directly smearing food on its fur, making the mouse to swim, or misting the mouse with water, therefore inducing self-grooming to clean and protect the body from foreign substances. A mouse with delayed self-grooming after being induced is assessed as having low motivation, which is similar to depression and apathy.<sup>2,4</sup>

In mice, body scratching with the hind legs may not be associated with other grooming activities. Scratching is a deeply complex motor action that reflects the perception of itchiness. Scratching might take several seconds and was started by elevating the hind paw to the area to be scratched. Various external stimuli, such as insects, parasites, allergens, and chemical irritants, can cause acute itch sensations that lead to scratching behavior.<sup>5,6</sup> House dust mites (HDM) are the most prevalent aeroallergen associated in people with allergy, particularly *Dermatophagoides pteronyssinus*, *Dermatophagoides farina*, and *Blomia Tropicalis*.<sup>7</sup> House dust mites have natural tendency for humid and warm environment.<sup>7,8</sup> Indonesia, as a tropical country with hot and humid environment, can be regarded as an ideal place for HDM to grow, with the most prevalent HDM being *Dermatophagoides pteronyssinus*.<sup>9</sup> The allergen with the highest positive SPT result among atopic dermatitis (AD) patients in the Dermatology and Venereology Outpatient Clinic at the Dr. Soetomo General Academic Hospital Surabaya, Indonesia, between 2017 and 2019 was HDM allergen, and half of the patients had a history of HDM allergy.<sup>10</sup>

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Atopic dermatitis is a chronic skin inflammation and cause several skin manifestations such as pruritus, dry skin, or erythema. Precipitating factors might make AD symptoms worse and lead to the condition relapsing. Allergen exposure, environmental factors and emotional factors can affect the relapse of AD.<sup>11</sup> Chronic itching, skin infections, sleep disorders, and

growth disorders can reduce the quality of life of AD patients and their parents.<sup>12</sup> People with atopic dermatitis were more susceptible to depression and anxiety, despite the severity of the disease<sup>13</sup>, which might result in self-grooming behavior changes. This pilot study compared grooming and scratching behavior between mice that exposed to allergen and placebo as well as the correlation between the two behaviors.

## **Methods**

### ***Animals and Treatment***

This experimental pilot study was conducted before the study of HDM immunotherapy in AD mouse model. This study involved six to eight weeks old male BALB/c mice were divided randomly into 2 groups, 8 mice each. The first group underwent the treatment of patch and spray with HDM allergen, while the second group was control group which was given NaCl 0.9% as placebo instead of HDM allergen. The patch was placed to the back of each mouse with 1x1cm gauze and hypoallergenic tape after shaving on day 1. The patch was left for one week and was removed on day 7. The spray was given to each group of mice with a nebulizer for 30 minutes every day since day 1 until day 7. The concentration of HDM allergen used in the study was 100µg for patch and 10<sup>-6</sup>µg for spray. The assessment for grooming and scratching was done on day 8. Observation and recording of each mouse were done using standard phone camera (1280x720 pixels and 29 frame per second) for 5 minutes in individual cage made for observation.

### ***Grooming behavior***

Grooming behavior assessed in this study included total grooming chain with cephalocaudal or head-to-body pattern

(hereinafter referred to as chain patterned grooming), grooming with non-cephalocaudal pattern (hereinafter referred to as non-chain patterned grooming), interrupted grooming bouts, body grooming, and total grooming duration. The chain patterned grooming must follow the sequence of cephalocaudal pattern starting from paw or nose grooming, face grooming, head grooming, and body grooming. Meanwhile the non-chain patterned grooming was counted when a mouse had any other sequence rather than the chain patterned grooming. Interrupted grooming bouts were counted when there were incomplete grooming sequences. When the mouse stopped grooming for more than 5 seconds, it would be counted as different bout. Total grooming duration was counted manually using stopwatch and presented in seconds.<sup>2,3</sup>

### ***Scratching behavior***

The counting of one scratch bout started from when the mouse lifted its hind leg towards a body area and ended when the hind leg was put back down. Any activity of the hind leg to the mouse's body surface was counted as scratching rather than grooming, except when the hind leg was put into the mouth, which is considered as grooming to remove the debris from the hind paw.<sup>14,15</sup>

### ***Statistical analyses***

The data were collected and analyzed with SPSS (ver. 26, IBM) and Prism (ver. 8, GraphPad Software) for visualization. Mann Whitney U test and Spearman correlation were used for the comparative and correlation analyses, respectively, as the data did not show normal distribution. The agreement between two independent researchers for grooming and scratching evaluation was assessed with Cohen's Kappa. Cohen's Kappa coefficient ( $\kappa$ ) of 0.61-

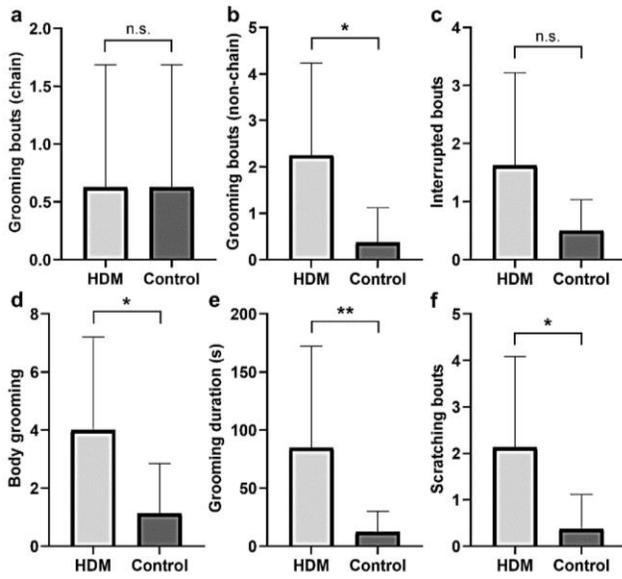
0.80 indicates substantial agreement and 0.81-1.00 indicates almost perfect agreement.<sup>16</sup> A *p* value of < 0.05 was considered as statistically significant.

**Ethical Clearance**

This study ethical clearance had been approved by the Ethical Committee from Faculty of Veterinarian Medicine Universitas Airlangga (No. 2.KE.11.09.2021).

**Results**

The amount of grooming activity (number of bouts and time spent grooming) and number of non-chain (non-cephalocaudal pattern) grooming as well as the scratching bouts were higher in HDM allergen group compared to control group.



**Figure 1** The amount of grooming and scratching behavior of both groups, results are presented in mean±SD. The chain patterned grooming had the same amount on both groups (a) while the interrupted bout of grooming was higher in HDM group (c), these results were not statistically significant. The non-chain patterned grooming (b), the body grooming (d), the total duration of grooming activity (e), and the total scratching bouts (f) were significantly higher in HDM group compared to control group.

\* *p* < 0.05; \*\* *p* < 0.01; n.s. not significant.

The number of chain (cephalocaudal pattern) were the same between two groups and interrupted grooming was found to be slightly higher in the HDM group. After further analysis, it was found that the difference between two groups was significant for the scratching bouts (*p*=0.017), non-chain patterned grooming (*p*=0.022), body grooming (*p*=0.014), and grooming duration (*p*=0.008). The data from the two groups can be observed in **Figure 1**.

The agreement between two independent researchers for grooming and scratching evaluation was assessed for each item separately. Agreement for chain patterned grooming had substantial agreement ( $\kappa=0.676$ , *p*<0.001), non-chain patterned grooming had moderate agreement ( $\kappa=0.565$ , *p*<0.001), interrupted grooming bouts had almost perfect agreement ( $\kappa=0.911$ , *p*<0.001), body grooming had substantial agreement ( $\kappa=0.778$ , *p*<0.001), grooming duration had moderate agreement ( $\kappa=0.535$ , *p*<0.001), and scratching behavior had substantial agreement ( $\kappa=0.743$ , *p*<0.001).

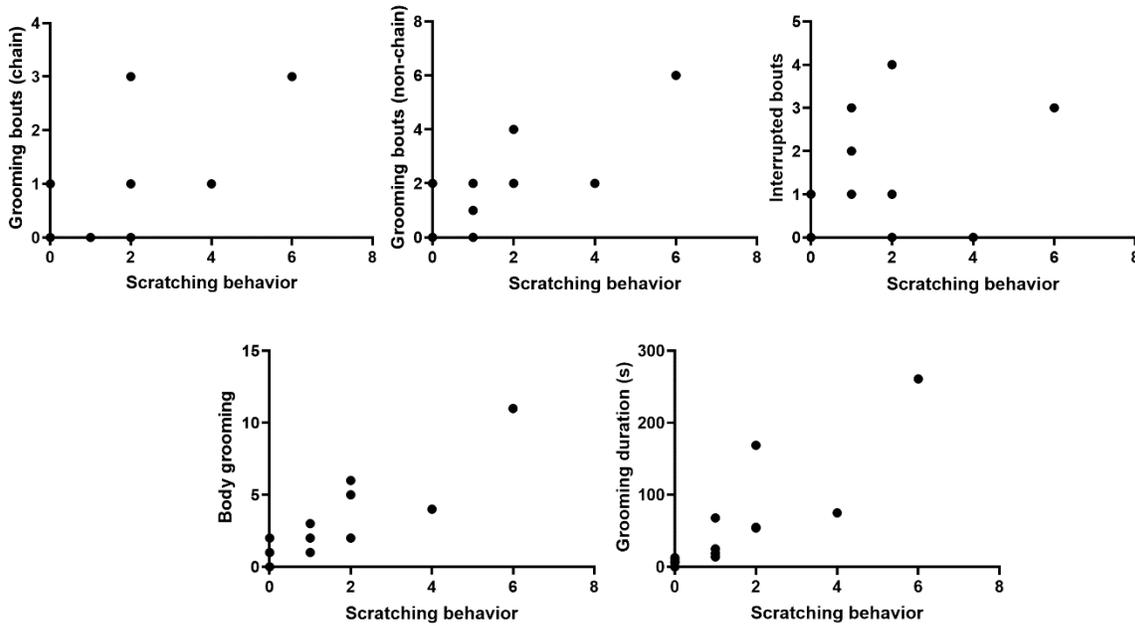
The correlation between grooming and scratching behavior was analyzed with Spearman correlation test. The value of spearman correlation coefficient (*r*) describes the strength of the correlation and can be interpreted as followed: very weak (0.00-0.19), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79) and very strong (0.80-1.00).<sup>17</sup> As shown in **Table 1**, the correlation of total grooming duration and scratching bouts, non-chain patterned grooming and scratching bouts, as well as body grooming and scratching bouts had high correlation (*p*<0.001). The scatter plots of these data can be observed on **Figure 2**. Meanwhile, the correlations between chain patterned grooming and interrupted bouts to scratching bouts were low and not significant.

**Table 1** Correlation of grooming and scratching behavior.

	<i>r</i>	<i>p</i>
Correlation of chain patterned grooming and scratching bouts	0.483	0.058
Correlation of non-chain patterned grooming and scratching bouts	0.769	0.000*
Correlation of interrupted bouts and scratching bouts	0.453	0.078
Correlation of body grooming and scratching bouts	0.863	0.000*
Correlation of grooming duration and scratching bouts	0.922	0.000*

r = spearman correlation coefficient.

\* statistically significant.



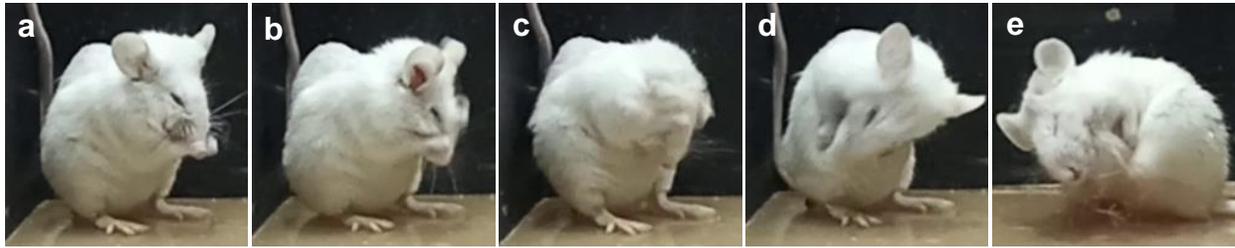
**Figure 2** The scatter plots of scratching bouts and other variables, such as (a) chain grooming bouts, (b) non-chain grooming bouts, (c) interrupted grooming bouts, (d) body grooming, and (e) grooming duration.

**Discussion**

Grooming behavior serves the vital purpose of caring for and protecting the body's surface.<sup>3</sup> In rodents, a typical self-grooming syntactic chain serially combines 20 or more grooming motions into four separate, predictable phases that follow the same cephalocaudal pattern. The syntactic chain pattern accounts for 10-15% of all reported grooming behavior in rodents, with the rest following less predictable sequence. More flexible 'non-chain' grooming (i.e., flexibly ordered sequences of strokes or licks that are not syntactic chains) accounts for 85-90% of all grooming behaviors.<sup>1</sup> The chain patterned grooming consists of 4 phases or elements and is executed in perfect sequence. Bilateral strokes

by the forepaws to the mouth of nose (paw or nose grooming) is the initial phase of the chain, followed by forepaws strokes around the mystacial vibrissae and below the eyes (face grooming), forepaws strokes backwards and upwards from behind the ear to the face (head grooming), and lastly strokes of the forepaws and licks to the body surface, including tail and genital (body grooming).<sup>1,2</sup>

Self-grooming activity can be induced by exposing the mouse into stressful situations which is the most common method used in studies about mouse self-grooming. Misting with water is one of the methods to induce artificial grooming, by making the mouse in direct contact with substance and inducing self-



**Figure 3.** The pattern of grooming behavior and scratching. The elements observed as grooming behavior in mice are consisting of (a) paw or nose grooming, (b) face grooming, (c) head grooming, and (d) body grooming. The chain patterned grooming is counted when these 4 elements were done in perfect sequence. Any other pattern consisting of these 4 movements but not done according to the sequence, it is counted as non-chain patterned grooming. The scratching movement is primarily done with the hind leg to the body area the mouse wants to scratch (e).

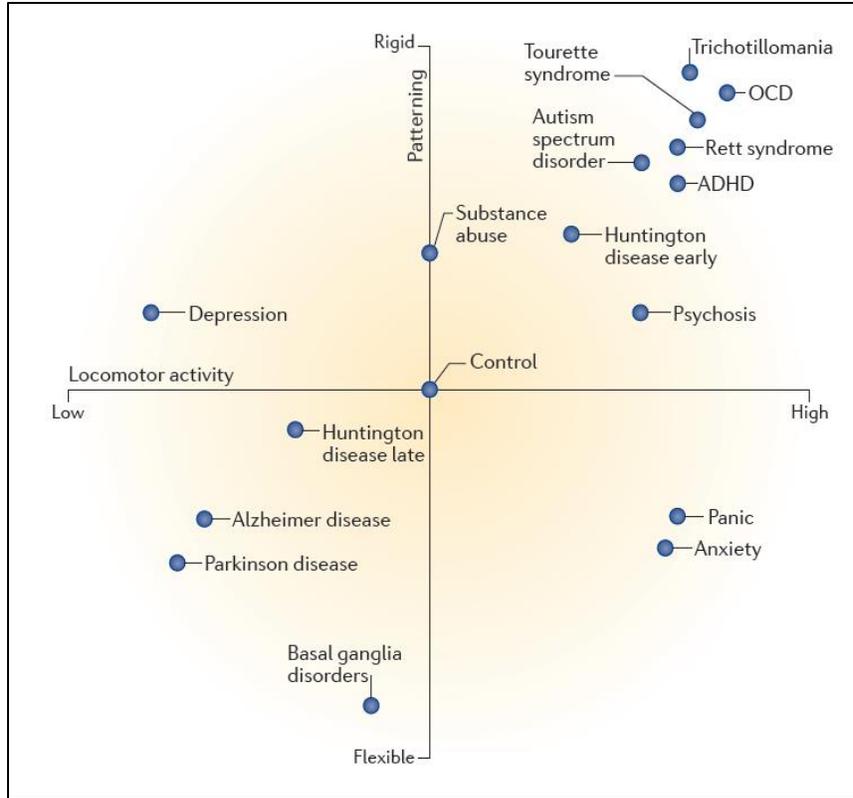
grooming to clean and protect the body from the foreign substances.<sup>2</sup> In this study the use of water spray, skin patch, and exposing the mice to HDM allergen could induce artificial grooming. Meanwhile, stressful situations might also influence the grooming behavior in this study. Stress-induced grooming can be observed with exposing the mice to new environment, bright light, or their predators.<sup>2</sup>

Scratching is a deeply complex motor action that reflects the perception of itchiness. Itchiness is an unsettling sensation of the skin that induces a desire to scratch. One count of scratching bout can last for several seconds and consist of a number of individual scratches. A scratching bout started by elevating the hind paw to the area of the body that needed to be scratched.<sup>6,15</sup> Skin-derived itch might be caused by inflammation, dryness, or damage of the skin. The itch stimuli cause cells in the skin including immune cells and keratinocytes, to release a variety of mediators, such as inflammatory mediators, neuromediators, and neuropeptides. These mediators bind to their receptors, causing itch-specific sensory neurons to be activated. The itch signals are transmitted through the dorsal root ganglion of the spinal cord, across the spinothalamic tract to the thalamus, and finally to the cerebral cortex from mechanically insensitive C-fibers (CMi) called histamine-dependent (histaminergic) or mechanically

sensitive C-type fibers (CMHs) called histamine-independent (nonhistaminergic) signaling pathways.<sup>18</sup>

When animal experience itching, it engages in recurrent scratching and licking behavior, which is stopped when the itch feeling fades. The amount and duration of scratching behavior are determined by the strength of itch stimuli. When an animal has a chronic itch, intermittent scratching and licking cycles occur. Depending on the frequency and severity of this behavior, the animal may develop a lesion at the scratching spot, which may begin with fur loss and progress to skin damage. The animal may eventually scrape its way through the epidermis to the dermis.<sup>6</sup>

The amount of grooming and scratching in HDM group were higher than control group in this study. House dust mites are the most prevalent aeroallergen associated in people with atopic disease such as atopic dermatitis, asthma, allergic rhinitis, and it might increase the risk to develop and exacerbate the disease.<sup>7</sup> House dust mites is often used as allergen to induce atopic dermatitis in mouse models.<sup>19,20</sup> In this study, the mice in HDM group were exposed with HDM allergen spray and patch for 7 continuous days. The increase in scratching behavior of mice in HDM group was statistically significant, which might be associated with itch sensation from the



**Figure 4.** Neuropsychiatric and neurodegenerative disorder with the expected grooming behavior in rodents. Rodent models such as mice or rats that are exposed to anxiogenic situations or medications might show increase in grooming behavior and more flexible pattern. Reprinted from Kalueff AV, *et al.*, 2016.<sup>1</sup> Copyright 2016 by Macmillan Publishers Limited.

development of dermatitis-like lesion on the mice's back skin. The grooming behavior might increase as a result of both artificial and stressful situations. The mice in HDM group were found to spent longer grooming time and in more non-chain patterned grooming, which was suspected to be associated with anxiety as showed in **Figure 4**. The increase of body grooming, which was expected because of the development of skin lesion on the back of the mice where the allergen patches were placed in HDM group, was also found in the study.

The statistical analyses found that the correlation between scratching behavior and non-chain patterned grooming, body grooming, as well as grooming duration were significant. The amount of scratching behavior had high correlation with non-chain patterned grooming, body grooming, and duration of grooming. The correlation between grooming and scratching behavior has not been analyzed further in previous studies, as the origin of the two behaviors have different

purposes and pathophysiology.<sup>21</sup> However, it is possible that they are associated with each other, especially in some cases with induced scratching or grooming behavior.

### Conclusion

House dust mite allergen increased the grooming and scratching behavior in mice compared to placebo. There were correlations between the scratching behavior and non-chain patterned grooming, body grooming as well as duration of grooming, which could indicate the anxiety or stressful situation caused by the itchiness from HDM allergen.

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