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FULL PAPER

Elevated cortisol content in dog hair with atopic dermatitis

Seol-Hee Park, Sun-A kim, Nam-Shik Shin and Cheol-Yong Hwang*)

The Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 151–742, Korea

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Abstract

Canine atopic dermatitis (CAD) is a chronic relapsing inflammatory skin disease occurring in 10% of the canine population. Although most studies have focused on the pathophysiological mechanism involved in CAD, the detrimental impact of CAD on quality of life has received only little attention. Hair cortisol analysis is becoming a valuable tool in monitoring chronic stress. To further validate this approach in CAD, we compared the hair cortisol concentration of atopic dogs with that of healthy conditioned dogs. The extent and severity of cutaneous lesions of atopic dermatitis were assessed according to modified CADESI-03 scores. In addition, skin barrier function was evaluated by measuring trans-epidermal water loss (TEWL) and stratum corneum conductance. The correlation between CAD severity and hair cortisol concentration was evaluated. The level of hair cortisol evaluated by ELISA assay showed that the atopic dermatitis group had significantly increased cortisol levels compared to that of the healthy control group. A significant positive correlation was identified between hair cortisol level and the CADESI score in CAD patients. The TEWL value of the cubital flexor of the forelimb in the atopic group was significantly higher compared to the healthy controls. These findings imply that the hair cortisol analysis can be an effective and objective biomarker in assessment of long-term stress of CAD patients.

Key Words: Atopic dermatitis, cortisol, dog, hair

1. Introduction

Atopic dermatitis (AD) is a chronically relapsing, inflammatory skin disease, characterized by typically distributed eczematous skin lesions, dry skin, intense pruritus, and a wide variety of pathophysiologic aspects in humans and dogs[6]. AD can lead to psychological stress[15], and it impacts the quality of life. In the field of veterinary dermatology, canine atopic dermatitis (CAD) is the second most common allergy in dogs occurring in 10% of the canine population[13].

*Corresponding author: Cheol-Yong Hwang, Faculty of Veterinary Medicine, Seoul National University, Gwan-ak ro 1, Seoul, Korea
Phone: +82 28801281. Fax: +82 28768662. E-mail: cyhwang@snu.ac.kr
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It is known that genetic, immunological, and environmental factors are involved in CAD[10]; however, the pathogenesis is not fully understood. A characteristic feature of AD is impairment of the epidermal skin barrier, which may increase transepidermal water loss (TEWL) and reduce skin hydration[2]. It leads to loss of the epidermal permeability barrier function and will drive AD development and progression.

Cortisol has long been considered a reliable physiological measure of the stress response in both humans[19,22] and other mammals[8]. Cortisol concentration can be measured in the blood, saliva, urine and hair[11,18]. Measurement of cortisol in the hair has some advantages against other types of samples. Hair sampling is noninvasive, painless and easy to store even for long periods. The cortisol in hair can reflect the long term chronic stress response over a period of months which is not influenced by circadian variation or the stress of sample collection[5,20]. A recent study has shown that cortisol immunoreactivity in hair was less variable compared to saliva and feces of dogs housed under constant conditions over 3 months[1]. This finding indicates hair might be a meaningful measure of baseline cortisol levels over time in dogs for which cortisol measurement in hair is a more practical approach to monitoring the effects of long-term stressors such as disease progression.

The mechanism on how cortisol enters the hair is unclear. Currently, one accepted mechanism is that cortisol enters the hair via passive diffusion from the blood that feeds the follicles and potentially from surrounding eccrine and sebaceous glands[14]. Another possibility is that the hair follicles themselves seem to produce cortisol locally[7]. Regardless of the above mentioned mechanism, cortisol concentrations in hair have been shown to reflect endocrine patterns[7].

The aim of this study was to evaluate the hair cortisol concentration in CAD. We expected elevated hair cortisol concentration in CAD related to chronic stressful physical conditions in CAD.

2. Materials and methods

2.1. Animals: Two groups of dogs were evaluated in this study. Dogs ($n = 21$) with atopic dermatitis were diagnosed based on their history, typical clinical signs, positive for serum allergen-specific IgE in serum and/or intradermal skin tests and by ruling out of other pruritic skin diseases. The clinical diagnostic criteria used in this study were proposed by Willems[23] and modified by Favrot *et al.*[4]. None of the dogs in this study had major clinical signs of illness other than the skin problem. Patients receiving systemic and/or topical glucocorticoid medications for 3 months preceding the study were all excluded from this study.

Dogs ($n = 5$) in the control group were healthy based on medical history and physical examination and had no clinical signs of skin problems.

All dogs were pets living in households, and written informed consent for the investigation was obtained from the owners of the dogs. All dogs underwent laboratory tests to determine their general medical condition.

2.2. Evaluation of lesion severity of CAD: The extent and severity of the cutaneous lesions were assessed according to modified CADESI-03 Scores[16,17].

2.3. Transepidermal water loss and skin hydration: Measuring trans-epidermal water loss (TEWL) and stratum corneum conductance were done to evaluate skin barrier function. TEWL was measured with an evaporimeter (VapoMeter: Defin Technologies Ltd. Kuopio. Finland) expressed as g/m^2/h. Skin hydration was measured by analyzing skin electrical impedance with a Corneometer (Corneometer CM 825: Courage & Khaqaka. Cologne. Germany) and expressed in arbitrary units. Prior to measuring the TEWL and skin hydration, the dogs were not permitted to exercise but were acclimated to the test room conditions. Measurements were taken in a

constant environment (ambient temperature of 22–26°C and 40–60% humidity) to minimize seasonal variation. Five consecutive measurements were done at following body sites: ventral neck, cubital flexor of the forelimb and inguinal area. All values are expressed as the median of five measurements to avoid inaccuracies.

2.4. Hair sampling: Hair was collected from the ischiatic region from within about 5 mm of the skin. Approximately 25–150 mg of hair was collected with commercially available pet grooming clippers. Each hair sample was placed into a plastic bag and stored at room temperature until the analysis.

2.5. Cortisol extraction from hair: Hair samples were washed twice in 5 ml of isopropanol by gentle rotation for 3 min. Hair was dried at room temperature for approximately 5 days and then minced into 1–3 mm length fragments inside a glass vial. Absolute methanol (2 ml) was added to the glass vial. A control sample consisting of a 2 ml aliquot of methanol was put into a glass vial without hair and also analyzed. The vials were gently agitated for 24 h at room temperature. After centrifugation (1500 g, 5 min), the supernatant was decanted, and the organic solvent was evaporated at 60°C for 3 h. Extracts were re-dissolved in 250 μl of assay buffer from the cortisol assay kit.

2.6. Cortisol assay: Cortisol was measured with the Salimetrics EIA (enzyme immunoassay) kit for salivary cortisol (Salimetrics, State college, PA, USA) previously described (Bennett at al., 2010). According to the manufacturer's guideline, cross-reactivity of the antibody with other steroids is less than 1% except for dexamethasone at 19.2%. The minimum concentration of cortisol detectable in the assay was 0.003 μL/dL. The mean intra- and inter- assay variation was 9.52% and 14.75%, respectively. Intra- and inter-assay CVs were calculated using cortisol concentrations from the kit standards.

2.7. Statistical analysis: Data were expressed as mean ± standard deviation. The statistical analysis was performed using Welch-t test for comparing variables between groups, and a P value ≤ 0.05 was considered statistically significant. The atopic dermatitis group was separated into mild, moderate, and severe disease state categories based on the classification methods of the reference article (Mild = CADESI 16–59, Moderate = CADESI 60–119, Severe = CADESI > 120)[17]. Linear regression was performed to assess the association between CADESI score and hair cortisol level.

3. Results

3.1. Subjects

Twenty one dogs with atopic dermatitis were enrolled in this study. The mean age of the atopic group was 7 ± 0.75 years. Nine females (three entire and six spayed) and twelve males (one entire, eleven neutered) were included in the atopic group. Breeds included Shih Tzu ($n = 8$), Poodle ($n = 3$), Beagle ($n = 2$), Yorkshire terrier ($n = 2$), Cocker spaniel ($n = 2$), Miniature Schnauzer ($n = 1$), Pekinese ($n = 1$), Maltese dog ($n = 1$) and Cavalier King Charles Spaniel ($n = 1$).

Five healthy dogs were included as the control group in this study. The mean age of the control group was 3 ± 1.03 years and comprised of four males and one female. Breeds included Shih Tzu Poodle ($n = 1$), Welsh corgi ($n = 1$), Norfolk terrier ($n = 1$) and one mixed breed dog ($n = 1$). Significant signs of systemic illness were not detected by physical examination and serum chemistry analyses.

3.2. Hair cortisol levels were elevated in patients with CAD

The level of hair cortisol evaluated by ELISA assay showed that the atopic dermatitis group had a significantly increased cortisol level (16.25 ± 2.86 pg/mg) compared to that of the

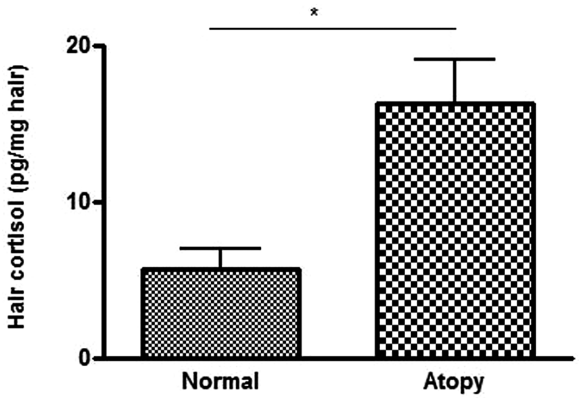


Fig. 1. Cortisol levels in hair in dogs with atopic dermatitis (n = 21) and in healthy dogs (n = 5). Hair cortisol levels were higher in the CAD group compared to control group.

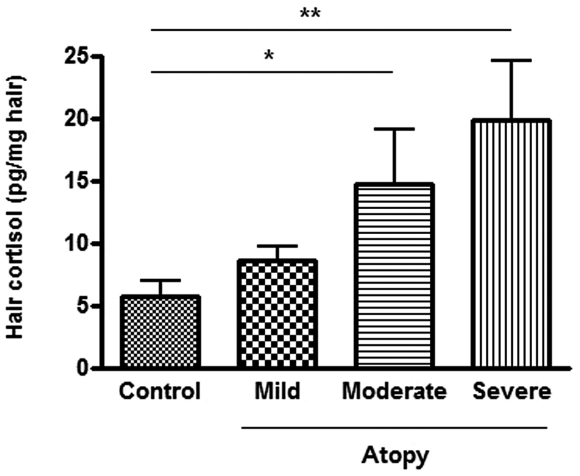


Fig. 2. Hair cortisol contents and disease state of atopic dermatitis. Hair cortisol levels in the moderate and severe atopic groups were significantly higher compared to healthy control.

healthy control group (5.65 ± 1.28 pg/mg) (Fig. 1, $P < 0.05$).

The atopic dermatitis group was separated into mild, moderate, and severe disease state categories according to the proposed cut-off value[17] of CADESI score. Mild CAD group include 4 dogs, moderate CAD group include 6 dogs and severe CAD group include 11 dogs, respectively. Although there was no obvious change in the hair cortisol level in the mild atopic groups, the hair cortisol level in the moderate and severe groups showed significant elevation compared to that of the healthy control group (Fig. 2). Mean hair cortisol levels of the atopic groups were 8.56 ± 1.1, 14.76 ± 4.32 and 19.85 ± 4.74 pg/mg in the mild, moderate and severe groups of CAD, respectively. A significant positive correlation was identified between hair cortisol levels and CADESI score in the canine atopic dermatitis patient seen in Fig. 3 ($r = 0.433$, $P \leq 0.05$).

3.3 Impaired skin barrier function in severe CAD

TEWL was measured from 3 anatomical body sites. The TEWL value of the cubital flexor of the forelimb in the atopic group (148.3 ± 47.59) was significantly higher compared to the healthy control (32.1 ± 1.4) ($P < 0.05$). The TEWL of the ventral neck in the atopic group (161.5 ± 55.47) was also increased compared to the healthy control (41.75 ± 5.15), but the difference was not statistically significant ($P = 0.52$). There was no significant difference in skin hydration between the groups.

4. Discussion

The main finding of our study is that cortisol levels in hair are significantly elevated in canine atopic dermatitis patients, especially in the groups with moderate and severe CAD disease state. Hair cortisol levels and clinical severity of CAD were positively correlated. Our results indicate that the hair cortisol levels might be increased in response to chronic physical discomfort from CAD (i.e., itchy, dry and inflamed skin conditions). Some studies found no effect of age and gender on the cortisol levels in hair[12]. In our study, mean age of the atopic group was 7 ± 0.75 years and that of control group was 3 ± 1.03. This may theoretically be explained by the clinical feature of CAD. Most of affected dogs experiencing the first sign of CAD before 3 years of age and underwent recurrent, chronic course of CAD with their ages[4].

The modified CADESI-03, used in this study, is an objective disease severity scale that has

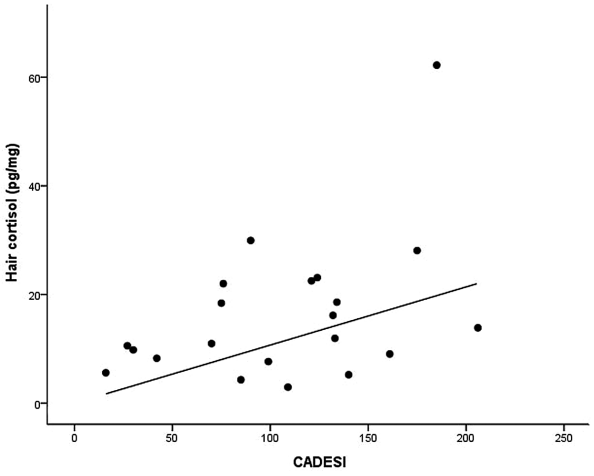


Fig. 3. Comparison of individual levels of hair cortisol and CADESI score in dogs with atopic dermatitis. A positive and statistically significant correlation was found and a line of best fit is plotted.

been accepted as a validated tool for assessing canine atopic dermatitis disease severity[16]. This study revealed that the clinical severity of CAD (i.e. CADESI score) was related with the hair cortisol level. The clinical severity of CAD was also checked with TEWL and stratum corneum conductance. The loss of the barrier function of the SC is involved in the development and aggravation of atopic dermatitis. In this study, impairment of the skin barrier function in the severe atopic dog group was detected with increased TEWL values although the level of skin hydration was not significantly decreased in CAD. A study by Shimada *et al.*, showed that the TEWL value was elevated both in lesional and non-lesional skin of CAD which was correlated to the decreased level of ceramide content in the skin[21]. According to the results of a previous study, skin hydration is not significantly affected in non-lesional skin of CAD. Based on the aforementioned studies, it is reasonable to speculate that water permeability could be increased without concomitant diminishment of water capacitance in non-lesional skin of CAD. In this study, we did not differentiate lesion and non-lesion during sample collection. It could affect the results of the skin hydration measurements. In this regard, further studies will be needed.

Recently, several studies have been conducted to assess health related quality of life and disease severity of human atopic dermatitis patients. It has been reported that patients with atopic dermatitis had inferior scores on mental health and social function in the Dermatology Life Quality index. In addition, patient-assessed severity of atopic dermatitis correlated with health related quality of life decrements[9]. A previous veterinary study, comparing the CADESI-03 score with the disease specific questionnaires form the owners, showed that disease severity of CAD was correlated with overall assessment related QoL[3]. The results we obtained were also consistent with the previous studies. We investigated the cortisol levels in hair, a biological marker for chronic stress and we found significant correlation between clinical severity of CAD and cortisol levels in hair. This result implies that the physical discomfort of CAD may affect QoL of affected individuals and as such we would suggest the hair cortisol analysis as an objective method to scale QoL of CAD patient. It is important to note that this new approach may provide additional information to the traditional objective clinical scoring systems.

As our knowledge, there are currently no effective and validate measures that include the clinical severity of CAD as well as other important aspects of disease severity, such as the quality of life in CAD. This pilot study is the first attempt to examine cortisol level in hair of CAD patient and assess its potential as a biomarker of stress. Measurement of corisol levels in hair has several advantages. Its collection is non-invasive and can provide a means of measuring long-term or basal cortisol secretions that are less sensitive to individual circadian patterns and momentary stressors. Nevertheless, future investigations are needed to elucidate the possibility of interrelationship between pharmacotherapy used to control of flare of CAD and hair cortisol levels.

Overall, our results suggest that hair cortisol analysis might be an effective and objective tool in assessment of the QoL of CAD patient.

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This research was conducted at the Dermatology clinic for small animal, Seoul National University, Korea. Participants were also recruited from regional hospital (Daul animal hospital, Ami animal hospital). No conflict of interest is declared by any of the authors.

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