Serum clusterin level in children with atopic dermatitis

In Suk Sol, M.D., Yoon Hee Kim, M.D., Kyung Eun Lee, Ph.D., Jung Yeon Hong, Ph.D., Mi Na Kim, M.Sc., Yun Seon Kim, B.Sc., Mi Seon Oh, B.Sc., Min Jung Kim, M.D., Seo Hee Yoon, M.D., Ph.D., Young A. Park, Ph.D., Kyung Won Kim, M.D., Ph.D., Myung Hyun Sohn, M.D., Ph.D., and Kyu-Earn Kim, M.D., Ph.D.

ABSTRACT

Background: *Atopic dermatitis (AD) is a chronic inflammatory skin disease. Clusterin is a sensitive cellular biosensor of oxidative stress and has been studied as a marker to assess inflammatory diseases. The clusterin levels in AD have not been evaluated thus far.*

Objective: *We evaluated serum clusterin levels in children with AD and assessed the relationship between serum clusterin levels and the severity of AD.*

Method: *The study enrolled a total 140 children, of whom 100 had AD (n = 100) and 40 were healthy (n = 40). The severity of AD was scored by using the SCORing Atopic Dermatitis (SCORAD). Total serum immunoglobulin E and specific immunoglobulin E levels against egg whites, cow's milk, peanuts, soybeans, wheat, and Dermatophagoides farinae were measured. Clusterin levels in serum were measured by enzyme-linked immunosorbent assay.*

Results: *The mean (interquartile range) age of the children was 5.1 years (1.3–8.4 years), and 92 (69.3%) of the children were boys. The mean (standard deviation) SCORAD index was 50.4 ± 17. The mean (standard deviation) clusterin level of children with AD was higher than that in the healthy control group children (148.13 ± 4.3 pg/mL versus 144.85 ± 5.1 pg/mL; p = 0.001). Serum clusterin levels were correlated with the SCORAD index (r = 0.327, p = 0.002).*

Conclusions: *The serum clusterin level was higher in children with AD than in the healthy control group and increased with the severity of AD. Serum clusterin may be a candidate molecule that reflects AD and its severity.*

(Allergy Asthma Proc 37:335–339, 2016; doi: 10.2500/aap.2016.37.3953)

Atopic dermatitis (AD) is a chronic or chronically relapsing inflammatory skin disease characterized by typically distributed eczematous skin lesions.[1] AD affects >20% of children in developed nations and entails an important decline of their quality of life.[2] Most cases of AD occur before 5 years of age, and up to 60% of these cases occur within the first year of life.[3] The diagnosis of AD relies exclusively on clinical features because there is no specific laboratory or histologic diagnosis for AD.[4]

Although the pathogenesis of AD has been focused on both skin barrier defects of the stratum corneum and immunologic dysregulation, the underlying mechanisms of skin inflammation in AD have not been completely understood.[5,6] At present, gene-environment interactions and environmental factors that affect skin barrier function have been studied as the main causes and pathogenesis of AD.[2,7,8] Results of some studies indicated that oxidative stress is implicated in the pathogenesis of AD based on an increase in urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels, a marker of oxidative stress, in children with AD or AD exacerbation.[6,9]

Clusterin is a heterodimeric glycoprotein that comprises 449 amino acids and has a molecular weight of ~80 kDa.[10] Clusterin is present in most human organs and is found in numerous physiologic fluids, including semen, urine, breast milk, cerebrospinal fluid, and plasma.[11,12] Stress-induced transcription of clusterin mediated by the heat shock transcription factor 1 results in its upregulation in response to a variety of stress conditions, including ischemia, neuronal injury, aging, and oxidative stress.[13] Clusterin is a widely distributed protein with many functions, including cellular protection in response to oxidative stress.[14]

Studies investigated the relationship between clusterin levels and oxidative stress or inflammatory disease.[15,16] Although there have been studies regarding clusterin's involvement in a variety of diseases, particularly oxidative or inflammatory diseases, the role of clusterin in AD has not yet been examined. The aim of this study was to compare serum clusterin levels in children with AD and in healthy children, and to assess

From the Department of Paediatrics, Severance Hospital, Institute of Allergy, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2011-0024036) and a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the Ministry of Health and Welfare, Republic of Korea (grant HI14C0234)

The authors have no conflicts of interest to declare pertaining to this article

Address correspondence to Myung Hyun Sohn, M.D., Department of Pediatrics, Institute of Allergy, Severance Children's Hospital, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea

E-mail address: mhsohn@yuhs.ac

the relationship between serum clusterin levels and the severity of AD.

METHODS

Patients and Study Design

We evaluated children who visited the allergy clinic for AD or for a general health workup at Severance Children's Hospital between June 2010 and November 2014. Children who were enrolled had no symptoms of other allergic disease such as asthma or allergic rhinitis besides AD. Children with AD fulfilled the revised Hanifin and Rajka criteria.[17] Disease severity was assessed by using the SCORing Atopic Dermatitis (SCORAD) index.[18] Children with AD were subdivided into two groups, mild-to-moderate AD (SCORAD score $\leq$ 50) and severe AD (SCORAD score $>$ 50).[19] Healthy controls had no history of AD or other allergic disease, such as asthma, allergic rhinitis, or inflammatory disease.

At first visit, blood samples were obtained from all the children. Peripheral white blood cells, eosinophil counts, and neutrophil counts were determined by using a hematology analyzer (NE-8000; Sysmex, Kobe, Japan). Total immunoglobulin E (IgE) and specific IgE levels were measured by using the Pharmacia CAP assay (Uppsala, Sweden). A specific IgE test was performed with six common allergens in Korea, including egg whites, cow's milk, peanuts, soybeans, wheat, and *Dermatophagoides farinae*. Atopy was defined as a specific IgE level of $\geq$0.7 KUa/L against more than one allergen or a total IgE level of 150 IU/mL. This study was approved by the institutional review board of Severance Hospital, and written informed consent was obtained from participants and their parents.

Measurement of Serum Clusterin Levels

At first visit, blood samples were taken from all the subjects to measure their clusterin level. After centrifugation at 400 $\times$ *g* for 10 minutes, the supernatants were then stored at -70°C for the subsequent assay of clusterin levels. Serum clusterin levels were measured by using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Statistical Analyses

All the statistical analyses were performed by using SPSS 20 (SPSS, Inc., Chicago, IL). Normally distributed data were analyzed by using one-way analysis of variance and the Student *t*-test; non-normally distributed data were analyzed by using the Mann-Whitney *U* test. Numerical parameters with a non-normal distribution (blood eosinophil count and serum total IgE) were log-transformed (natural logs) before analysis. Correlations between clusterin levels and SCORAD scores, total serum eosinophil counts, or total IgE levels were evaluated by using the Spearman's rank correlation. A result was deemed significant at $p < 0.05$.

RESULTS

Characteristics of Subjects

This study included 140 children (100 with AD and 40 normal controls) with a median (interquartile range [IQR]) age of 5.1 years (1.3–8.4 years), and the male to female ratio was 2.3:1. Median (IQR) blood eosinophil counts were higher in children with AD than in controls (650 μL [360–1093 μL] versus 145 μL [83–238 μL], $p < 0.001$). Children with AD also had a higher median (IQR) total IgE compared with controls (511.5 IU/mL [206–1637 IU/mL] versus 32.6 IU/mL [20–68 IU/mL], $p < 0.001$). The median (IQR) specific IgE level was 3.0 kU/L (0.06–26.9 IU/mL) for egg whites, 0.99 IU/mL (0.37–7.1 IU/mL) for milk, and 0.42 IU/mL (0.07–3.83 IU/mL) for peanut in children with AD. Of those with AD, there were 24 children with mild-to-moderate AD (median [IQR] age 1.5 years [0.6–5.4 years]; 16 boys [66.7%]) and 61 with severe AD (median [IQR] age, 3.2 years [0.8–6.9 years]; 47 boys [77.0%]) (Table 1). Patients with severe AD had a higher median (IQR) total blood eosinophil count and total IgE level compared with the those with mild-to-moderate AD (840 mL [535–1290 mL] versus 500 mL [330–720 mL], $p = 0.025$; and 715 IU/mL [316–2369 IU/mL] versus 337 IU/mL [86–740 IU/mL], $p < 0.001$, respectively (Table 1).

Clusterin Levels in AD and in Healthy Controls

Mean (SD) serum clusterin levels were higher in children with AD than in the healthy controls (148.13 $\pm$ 4.3 ng/mL versus 144.85 $\pm$ 5.1 ng/mL; $p < 0.001$) (Fig. 1). Children with severe AD had higher mean (SD) serum clusterin levels than those with mild-to-moderate AD (149.25 $\pm$ 3.2 ng/mL versus 147.07 $\pm$ 5.0 ng/mL; $p = 0.01$) (Fig. 1). There also were significant differences in clusterin levels between AD and controls, and between children with mild-to-moderate AD and children with severe AD after adjusting for differences in age.

Correlation of Serum Clusterin Levels with Disease Severity

Serum clusterin levels were significantly correlated with the SCORAD index ($r = 0.327$, $p = 0.002$) (Fig. 2).

Correlation of SCORAD with Blood Eosinophil Counts and IgE Levels

The SCORAD index in children with AD showed significant correlations with blood eosinophil counts and total IgE levels ($r = 0.360$, $p = 0.001$ and $r = 0.285$, $p = 0.008$, respectively) (Fig. 3, *A* and *B*).

Table 1 **Patient characteristics**

Characteristics	Control	Atopic Dermatitis (n = 100)	
	(n = 40)	Mild to Moderate (n = 51)	Severe (n = 49)
Age, median (IQR), y	8.3 (6.6–11.1)*	2.3 (0.8–5.4)	4.4 (0.7–6.9)
Boys, no. (%)	20 (50.0)	36 (70.6)	41 (83.7)
Eosinophils, median (IQR), μL	145 (83–238)*	500 (330–720)†	840 (535–1290)§
Total IgE level, median (IQR), IU/mL	32.6 (20–68)*	337.0 (86–740)†	715.0 (316–2369)§
SCORAD, mean (SD)		36.9 ± 8.9¶	63.5 ± 10.8

IQR = interquartile range; IgE = immunoglobulin E; SCORAD = Scoring Atopic Dermatitis; SD = standard deviation.
**$p < 0.01$ compared with mild-to-moderate atopic dermatitis by using the Kruskal-Wallis test and the Dunn method for multiple comparisons.*
†$p < 0.01$ compared with severe atopic dermatitis by using the Kruskal-Wallis test and the Dunn method for multiple comparisons.
§$p < 0.01$ compared with healthy controls by using the Kruskal-Wallis test and the Dunn method for multiple comparisons.
¶$p < 0.01$, as compared with severe atopic dermatitis when using the Mann-Whitney U test.

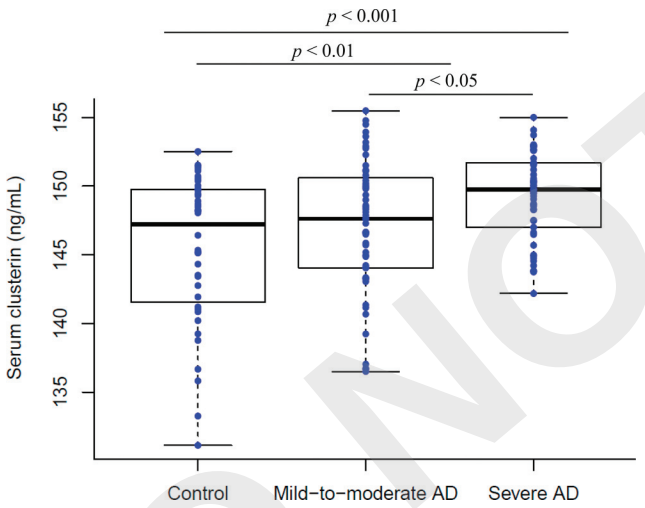

Figure 1. *Serum clusterin levels in patients with atopic dermatitis and controls according to severity.*

Correlation of Serum Clusterin Levels with Blood Eosinophil Counts and IgE Levels

In children with AD, serum clusterin levels showed significant correlations with total IgE levels (r = 0.434, $p < 0.001$) (Fig. 3, *C*) but not serum eosinophil counts.

DISCUSSION

The main finding of this study was that serum clusterin levels were increased in children with AD and correlated with disease severity. AD remains a clinical diagnosis and assessment because there is no objective, reliable biomarker to confirm the diagnosis of AD and to monitor control of the disease.[20] Skin is exposed to oxidative stress from endogenous and exogenous sources: endogenous sources include enzymes and inflammatory cells (*e.g.*, neutrophils, ischemia, inflammation), and endogenous sources include pollutants, ozone, chemicals, and ultraviolet irradiation.[21]

Previous studies sought to measure a specific urinary marker, 8-OHdG, one of the major oxidative DNA lesions induced by radical agents, as a biomarker for oxidative stress in children with AD.[6,9,22] The studies were conducted with the hypothesis that impaired homeostasis of oxygen and/or nitrogen radicals may participate in the development and progression of inflammatory processes in childhood AD.[6,9,22] Traditionally, reactive oxygen species have been known to be implicated in the progression of various kinds of inflammatory diseases, including AD.[13] Cellular damage by increased oxidative stress may be reflected in DNA, proteins, and lipids.[23] Results of one study indicated that excess reactive oxygen species generated allergic inflammatory sites and accelerated oxidative damage to DNA and other macromolecules in the skin of patients with AD.[24]

Because an increase in clusterin levels reflects a state of oxidative stress or accompanying inflammation in related diseases,[23] our findings indicated that oxidative stress in tissues of children with AD was more than that of healthy controls, similar to the results of previous studies.[6,9] Clusterin might play a role similar to that of 8-OHdG in previous studies[6,9], in which it was increased in oxidative stress and children with AD. Moreover, serum clusterin levels were also increased according to the severity of AD, unlike previous studies[6] in which 8-OHdG was not correlated with severity according to SCORAD.

The reported serum biomarkers for severity of AD were eosinophilic cationic protein, total IgE, soluble interleukin-2 receptor, interleukin 17, interleukin 23, endothelin-1, and thymus and activation-regulated chemokine.[25–28] The current study showed that the SCORAD index was correlated with total IgE levels,

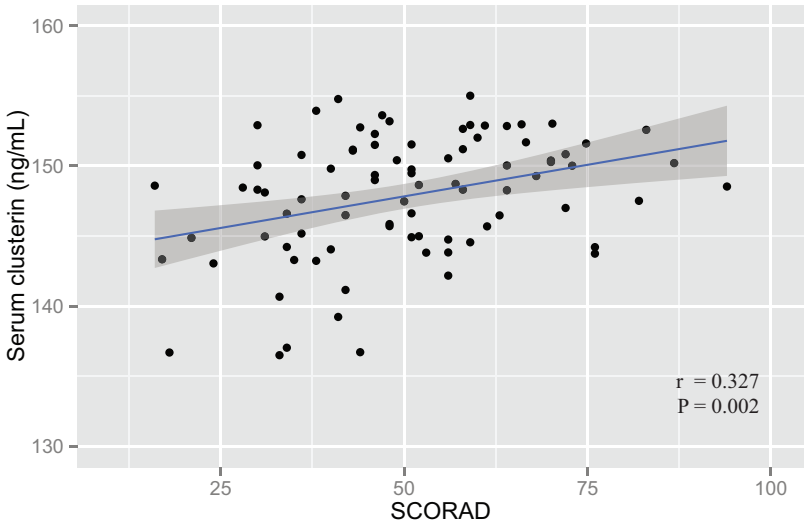

Figure 2. *Correlation between serum clusterin levels and severity of atopic dermatitis.*

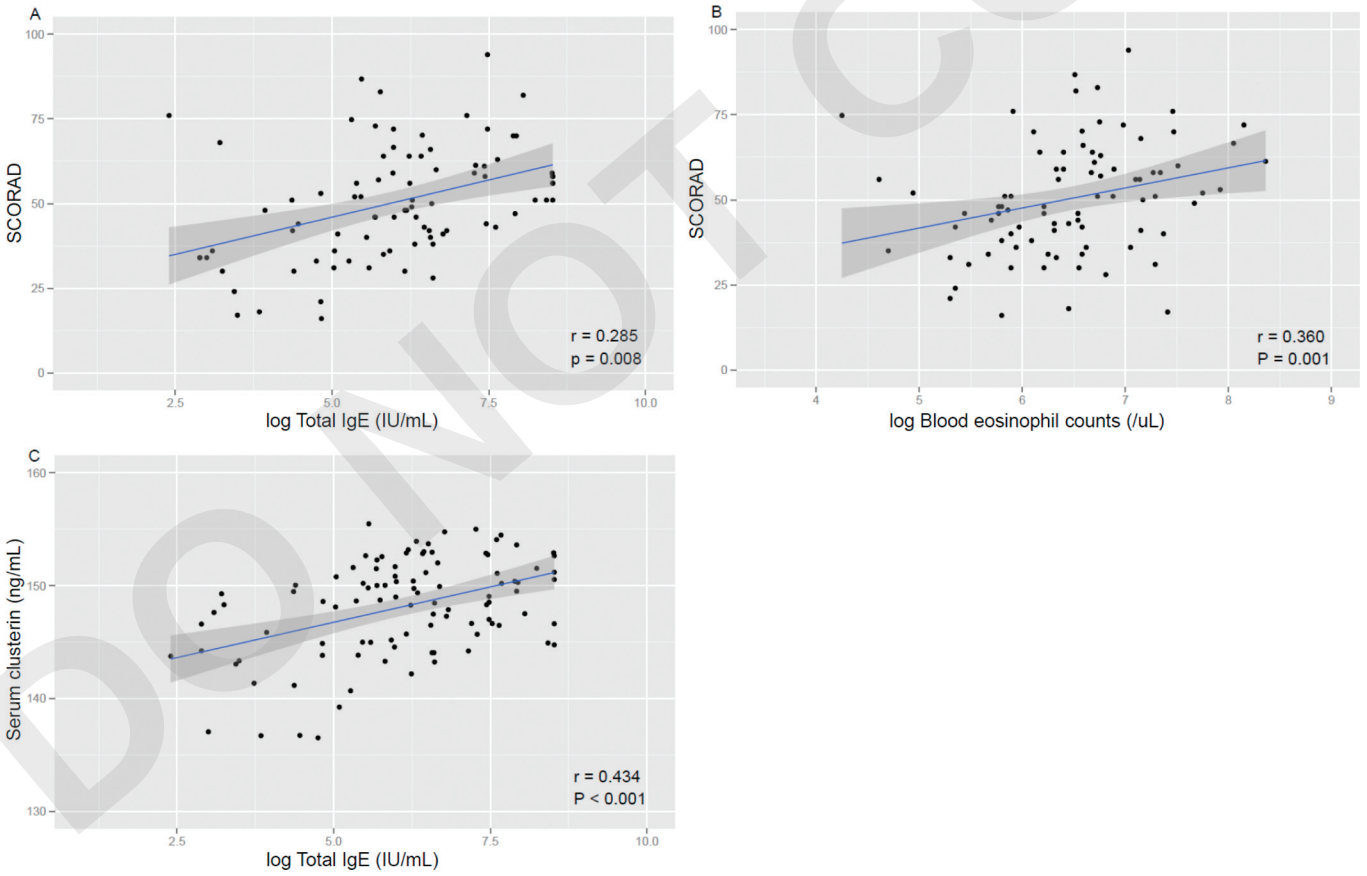

Figure 3. *Correlation of serum clusterin and Scoring Atopic Dermatitis (SCORAD) index with serum eosinophil count and total immunoglobulin E (IgE). (A) The SCORAD index was significantly correlated with total IgE (log transformed) in children with atopic dermatitis (AD). (B) Correlation between the SCORAD index and the blood eosinophil count (log transformed). (C) Correlation between serum clusterin level and total IgE level (log transformed).*

and serum clusterin levels in children with AD were significantly correlated with not only total IgE level but also the SCORAD index. Our results demonstrated that serum clusterin levels might be useful markers for disease severity in childhood AD.

Recently, many studies identified that AD prevalence is positively associated with traffic-related air pollution, nitrogen dioxide, and nitric oxide.[29–31] The skin barrier related to the main pathogenesis of AD seems to be directly damaged by oxidative stress ini-

tiated by external pollutants.[32] These findings corroborate our results of significantly higher clusterin levels in AD and its correlation with the severity of AD based on the SCORAD index.

CONCLUSION

Serum clusterin levels were significantly elevated in patients with AD, particularly in those severely affected by the disease, compared with patients with mild-to-moderate AD and healthy controls. The serum clusterin levels were significantly correlated with the clinical severity of AD. Therefore, the serum clusterin level could be considered as a candidate molecule in the diagnosis of AD and an objective indicator for evaluating AD disease activity.

REFERENCES

1. Leung DY. Atopic dermatitis: New insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 105:860–876, 2000.
2. Flohr C, and Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy 69:3–16, 2014.
3. Montes-Torres A, Llamas-Velasco M, Perez-Plaza A, et al. Biological treatments in atopic dermatitis. J Clin Med 4:593–613, 2015.
4. Weidinger S, and Novak N. Atopic dermatitis. Lancet 2015. (Epub ahead of print September 11, 2015.)
5. Bieber T. Atopic dermatitis. N Engl J Med 358:1483–1494, 2008.
6. Omata N, Tsukahara H, Ito S, et al. Increased oxidative stress in childhood atopic dermatitis. Life Sci 69:223–228, 2001.
7. De Marchi F, Piacentini GL, Piazza M, et al. Correlation of skin barrier impairment in atopic dermatitis with aeroallergen sensitization. Allergy Asthma Proc 36:e127–e133, 2015.
8. Stelmach I, Bobrowska-Korzeniowska M, Smejda K, et al. Risk factors for the development of atopic dermatitis and early wheeze. Allergy Asthma Proc 35:382–389, 2014.
9. Tsukahara H, Shibata R, Ohshima Y, et al. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. Life Sci 72:2509–2516, 2003.
10. Won JC, Park CY, Oh SW, et al. Plasma clusterin (ApoJ) levels are associated with adiposity and systemic inflammation. PLoS One 9:e103351, 2014.
11. Jones SE, and Jomary C. Clusterin. Int J Biochem Cell Biol 34:427–431, 2002.
12. Rosenberg ME, and Silkensen J. Clusterin: Physiologic and pathophysiologic considerations. Int J Biochem Cell Biol 27:633–645, 1995.
13. Viard I, Wehrli P, Jornot L, et al. Clusterin gene expression mediates resistance to apoptotic cell death induced by heat shock and oxidative stress. J Invest Dermatol 112:290–296, 1999.
14. Carnevali S, Luppi F, D'Arca D, et al. Clusterin decreases oxidative stress in lung fibroblasts exposed to cigarette smoke. Am J Respir Crit Care Med 174:393–399, 2006.
15. Wu ZC, Yu JT, Li Y, and Tan L. Clusterin in Alzheimer's disease. Adv Clin Chem 56:155–173, 2012.
16. Savkovic V, Gantzer H, Reiser U, et al. Clusterin is protective in pancreatitis through anti-apoptotic and anti-inflammatory properties. Biochem Biophys Res Commun 356:431–437, 2007.
17. Eichenfield LF, Hanifin JM, Luger TA, et al. Consensus conference on pediatric atopic dermatitis. J Am Acad Dermatol 49:1088–1095, 2003.
18. Severity scoring of atopic dermatitis: The SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 186:23–31, 1993.
19. Oranje AP, Glazenburg EJ, Wolkerstorfer A, and de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: The SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 157:645–648, 2007.
20. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: Section 1. Diagnosis and assessment of atopic dermatitis. J Am Acad Dermatol 70:338–351, 2014.
21. Trouba KJ, Hamadeh HK, Amin RP, and Germolec DR. Oxidative stress and its role in skin disease. Antioxid Redox Signal 4:665–673, 2002.
22. Sabatini L, Barbieri A, Tosi M, et al. A method for routine quantitation of urinary 8-hydroxy-2′-deoxyguanosine based on solid-phase extraction and micro-high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 19:147–152, 2005.
23. Trougakos IP, and Gonos ES. Chapter 9: Oxidative stress in malignant progression: The role of Clusterin, a sensitive cellular biosensor of free radicals. Adv Cancer Res 104:171–210, 2009.
24. Tsuboi H, Kouda K, Takeuchi H, et al. 8-Hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. Br J Dermatol 138:1033–1035, 1998.
25. Thijs JL, Nierkens S, Herath A, et al. A panel of biomarkers for disease severity in atopic dermatitis. Clin Exp Allergy 45:698–701, 2015.
26. Leonardi S, Cuppari C, Manti S, et al. Serum interleukin 17, interleukin 23, and interleukin 10 values in children with atopic eczema/dermatitis syndrome (AEDS): Association with clinical severity and phenotype. Allergy Asthma Proc 36:74–81, 2015.
27. Tsybikov NN, Petrisheva IV, Kuznik BI, and Magen E. Plasma endothelin-1 levels during exacerbation of atopic dermatitis. Allergy Asthma Proc 36:320–324, 2015.
28. Song TW, Sohn MH, Kim ES, et al. Increased serum thymus and activation-regulated chemokine and cutaneous T cell-attracting chemokine levels in children with atopic dermatitis. Clin Exp Allergy 36:346–351, 2006.
29. Morgenstern V, Zutavern A, Cyrys J, et al. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. Am J Respir Crit Care Med 177:1331–1337, 2008.
30. Sybilski AJ, Zalewska M, Furmanczyk K, et al. The prevalence of sensitization to inhalant allergens in children with atopic dermatitis. Allergy Asthma Proc 36:e81–e85, 2015.
31. Lee YL, Su HJ, Sheu HM, et al. Traffic-related air pollution, climate, and prevalence of eczema in Taiwanese school children. J Invest Dermatol 128:2412–2420, 2008.
32. Ahn K. The role of air pollutants in atopic dermatitis. J Allergy Clin Immunol 134:993–999; discussion 1000, 2014. □