Clinical significance of serum high-mobility group box 1 level in alopecia areata

Young Lee, MD,[a] Hae-Eul Lee, MD,[a] Jung-Min Shin, MS,[a] Kyung-Cheol Sohn, PhD,[a] Myung Im, MD,[a] Chang-Deok Kim, PhD,[a] Young-Joon Seo, MD,[a] Young-Ho Lee, MD,[b] and Jeung-Hoon Lee, MD[a]
Daejeon, Korea

Background: Alopecia areata (AA), a chronic, relapsing hair-loss disorder, is considered to be a T-cell-mediated autoimmune disease. High-mobility group box 1 (HMGB1), released by necrotic cells and in response to various inflammatory stimuli, is currently considered to be a significant target antigen in diverse autoimmune diseases.

Objective: We sought to investigate the clinical significance of serum HMGB1 levels in AA.

Methods: We compared levels of HMGB1 in scalp specimens from 7 patients with AA and 8 healthy control subjects and in blood samples from 45 patients with AA and 10 healthy control subjects. Moreover, we evaluated the correlation between HMGB1 level and clinical severity.

Results: Immunohistochemical staining of scalp tissues from patients with AA revealed higher HMGB1 levels than in healthy control subjects. In addition, serum HMGB1 levels in the AA group were generally higher, and showed concordance with the patients' clinical characteristics, including onset, hair-pull test results, and treatment response.

Limitations: The number of patients and healthy control subjects evaluated was small.

Conclusion: These results suggest that HMGB1 plays a significant role in the pathogenesis of AA, and that it is a promising predictor of prognosis and treatment response. Moreover, this study identifies a new potential therapeutic target for the treatment of AA. (J Am Acad Dermatol 2013;69:742-7.)

Key words: alopecia areata; autoimmune disease; high-mobility group box 1 protein.

Alopecia areata (AA) is the most frequent cause of inflammation-induced hair loss.[1,2] Although the pathogenesis of AA is poorly understood, accumulating evidence suggests that T cells and cytokines play an important role.[3] It has been hypothesized that AA develops in a previously healthy hair follicles because the collapse of constitutive immune privilege is coupled to T-cell-mediated attack of hair follicle autoantigens.[1,4] In addition to the observed associations between AA and various autoimmune disorders such as autoimmune thyroiditis and vitiligo, there is increasing evidence that AA is a tissue-specific autoimmune disease.[4-6] Cytokines contributing to the pathogenesis of these autoimmune disorders have been described. Recent studies have shown an association between high-mobility group

Abbreviations used:

AA:	alopecia areata
HMGB1:	high-mobility group box 1
IL:	interleukin

From the Departments of Dermatology[a] and Anatomy,[b] Research Institute of Medical Sciences, School of Medicine, Chungnam National University.
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Reprint requests: Jeung-Hoon Lee, MD, Department of Dermatology, Research Institute of Medical Sciences, School of Medicine, Chungnam National University, 33 Munhwa-ro, Daejeon 301-747, Korea. E-mail: jhoon@cnu.ac.kr.
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box 1 (HMGB1) and chronic inflammation and autoimmunity.[7]

HMGB1 is a ubiquitous and conserved protein located in all mammalian nuclei at high concentrations. It acts as a proinflammatory cytokine in both acute and chronic inflammatory conditions such as septic shock, acute lung injury, and rheumatoid arthritis.[8,9] It is actively released from lipopolysaccharide-, tumor necrosis factor-alfa–, and interleukin (IL)-1–activated monocytes and macrophages and from other cell types. HMGB1 can also be passively released from damaged dying cells during necrosis, and during the late phase of apoptosis. Extracellular HMGB1 exerts its biological actions by binding to cell-surface receptors such as receptor of advanced glycation end-products, Toll-like receptor 2, Toll-like receptor 4, and the intracellular receptor Toll-like receptor 9.[10] There is growing interest in the association of HMGB1 with autoimmune disorders, in which it serves as a significant target antigen. Increased HMGB1 expression has been detected in several autoimmune disorders, including systemic lupus erythematosus, Sjögren syndrome, and rheumatoid arthritis. In addition, regarding the cutaneous manifestations of autoimmune disease, studies have revealed increased expression of HMGB1 in systemic sclerosis and cutaneous lupus erythematosus.[9,11] AA is considered a tissue-specific autoimmune disease and so it is possible that HMGB1 may also contribute to the pathogenesis of AA.

There is currently no literature on the role of HMGB1 in the pathogenesis of AA. In this study, we compared HMGB1 levels in patients with AA and healthy control subjects and evaluated the correlation with clinical markers.

CAPSULE SUMMARY

- Although high-mobility group box 1 is considered a significant target antigen in various autoimmune diseases, its role in alopecia areata (AA) has not been comprehensively studied.
- High-mobility group box 1 levels were significantly higher in patients with AA compared with healthy control subjects and showed concordance with disease severity.
- High-mobility group box 1 may play a significant role in the pathogenesis of AA and may constitute a new potential therapeutic target for the treatment of AA.

METHODS

Patients

Scalp and blood samples were obtained from patients with AA and healthy control subjects in accordance with the ethical committee approval granted by Chungnam National University, Daejeon, Korea (institutional review board no. 201208-023). Those who had other types of illness, such as autoimmune diseases, other chronic diseases, or infectious conditions at the time of blood sampling, and those receiving systemic treatments such as immunosuppressive agents, were excluded. None of the healthy control subjects had a history of AA. Detailed clinical information regarding the clinical markers whose relationships with HMGB1 levels we wished to evaluate was obtained from all patients at the time of blood collection. The blood samples were centrifuged at 1000*g* (3000 rpm) for 15 minutes and then stored at −20°C or at −80°C before assaying. Each participant signed an informed consent form relating to their participation in this study.

Immunohistochemical analysis of skin samples

Paraffin sections of normal control and AA scalp were de-waxed, rehydrated, and then washed 3 times with phosphate-buffered saline. After treatment with proteinase K (1 mg/mL) for 5 minutes at 37°C, sections were treated with H_2O_2 for 10 minutes at room temperature, placed in a blocking solution (Dako, Carpinteria, CA) for 20 minutes, followed by reaction with the appropriate primary antibodies. Sections were incubated sequentially with peroxidase-conjugated secondary antibodies (Upstate, Lake Placid, NY) and visualized using a Chemmate Envision detection kit (Dako). HMGB1 antibody was purchased from Abcam (ab18256, Cambridge, MA). The intensity of HMGB1 only in dermis of scalp was analyzed using an image analyzer (i-solution, iMTechnology, Bucheon, Korea).

Enzyme-linked immunosorbent assay analysis of serum HMGB1 levels

Serum levels of HMGB1 were detected using an enzyme-linked immunosorbent assay kit (MyBioSource, San Diego, CA), according to the manufacturer's protocol.

Clinical assessment

To evaluate the correlation of clinical characteristics with HMGB1 level, we assessed several clinical markers. Disease type, initial hair-pull test result, disease onset, and treatment response were the clinical markers assessed.