Letter to the Editor

DOI: 10.5582/bst.2024.01084

Elevation of circulating DNAs of disease-associated cytokines in serum cell-free DNA from patients with alopecia areata

Soichiro Sawamura[§], Tselmeg M. Myangat[§], Ikko Kajihara[*], Katsunari Makino, Jun Aoi, Shinichi Masuguchi, Satoshi Fukushima

Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan.

SUMMARY Alopecia areata (AA) is an autoimmune disease characterized by damage to hair follicles and hair loss. Cell-free DNA (cfDNA) has recently received attention as a biomarker of various disorders including inflammatory skin diseases. In this study, we aimed to investigate the clinical significance of cfDNA and the circulating DNAs of disease-associated cytokines in AA patients. Serum samples were obtained from 63 patients with AA and 32 healthy controls (HC). Using droplet digital polymerase chain reaction, circulating C-X-C motif chemokine ligand (*CXCL*) 9, *CXCL10*, *CXCL11*, *C-X-C* motif chemokine receptor 3, interferon (*IFN*)-γ, interleukin (*IL*) -7, *IL-15*, and Janus kinase (*JAK*) 2 were detectable in both HC and AA patients. Among the detectable DNAs, copies of circulating *CXCL9*, *CXCL11*, *IL-15*, *IFN-γ*, and *JAK2* were significantly higher in AA patients than in HC. These results suggest that increased circulating DNA levels may reflect damage to hair follicles in AA patients.

Keywords liquid biopsy, chemokine, Janus kinase, digital PCR

To the Editor,

Alopecia areata (AA) is an autoimmune disease characterized by damage to hair follicles, resulting in the various levels of hair loss (*1*). Some pro-inflammatory cytokines have been shown to play key roles in the pathogenesis of AA (*2*). Circulating cell-free DNA (cfDNA) originates from apoptotic or necrotic cells and reflects the severity of cellular damage (*3*). Although cfDNA has recently received attention as a biomarker for the diagnosis and prognosis of various disorders including inflammatory skin diseases (*4,5*), the clinical significance of cfDNA in patients with AA remains unclear. In this study, we aimed to investigate the clinical significance of cfDNA in patients with AA and assess the circulating DNAs of cytokines associated with AA.

Serum samples were obtained from 63 patients with AA (all of them were diagnosed based on clinical presentation and pathological findings of skin biopsy), including 7 patients with patchy type, 31 with reticular type, 8 with ophiasis type, 11 with alopecia totalis, and 6 with alopecia universalis. Serum samples were collected from 32 healthy controls (HC). The clinical findings of the patients with AA were assessed at the time of serum sampling. cfDNA was isolated from the serum samples using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany). The cfDNA concentration was determined using a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The detection of circulating DNAs in cfDNA was performed using droplet digital polymerase chain reaction (ddPCR) according to our previous report (*5*). All probes used in this study were purchased from Bio-Rad (Hercules, CA, USA). All the samples were stored at -80°C before use. Institutional Review Board approval (No. 1452) and written informed consent were obtained in accordance with the principles of the Declaration of Helsinki.

Firstly, no significant difference was observed in the levels of total cfDNA between patients with AA (mean; 882.5 ± 857.7 ng/μL) and HC (mean; 908.5 ± 1260.8 ng/μL) (Table 1). Next, we attempted to detect circulating DNAs of patients with AA using ddPCR (Table 1). Although ddPCR showed that circulating C-X-C motif chemokine ligand (*CXCL*) 9, *CXCL10*, *CXCL11*, *C-X-C* motif chemokine receptor (*CXCR*) 3, interferon (*IFN*)-γ, interleukin (*IL*) -7, *IL-15*, and Janus kinase (*JAK*) 2 were detectable in both HC and AA patients, circulating *IL-2*, *JAK1*, *JAK3*, tyrosine kinase 2 (*TYK2*) were not detectable. Among the detectable DNAs, copies of circulating *CXCL9*, *CXCL11*, *IL-15*, *IFN-γ*, and *JAK2* were significantly higher in patients with AA than in HC. No significant relationships were observed between their levels and the clinical features such as age, sex, type,

Table 1. Serum cell-free DNA levels and circulating DNAs copies in patients with alopecia areata and healthy controls

Items	Healthy controls (n = 32)	Patients with alopecia areata (n = 63)	p-value
cell-free DNA levels (ng/mL ± SD)	908.5 ± 1,260.8	882.5 ± 857.7	0.238
circulating DNA levels (copies/mL ± SD)			
CXCL9	6,701.5 ± 11,138.8	7,647.0 ± 7,380.8	*0.015
CXCL10	8,706.6 ± 14,408.8	8,708.2 ± 8,013.0	0.103
CXCL11	5,702.2 ± 9,946.3	6,740.1 ± 7,390.4	*0.030
CXCR3	7,541.8 ± 13,470.7	7,198.7 ± 7,048.7	0.122
IFN-γ	6,634.6 ± 10,282.8	8,123.4 ± 7,847.0	*0.030
IL-7	9,850.4 ± 16,541.4	9,986.3 ± 8,834.0	0.060
IL-15	7,468.2 ± 12,480.2	8,183.2 ± 7,837.6	*0.038
JAK2	6,677.1 ± 10,658.2	7,922.4 ± 7,717.0	*0.041
IL-1	ND	ND	-
JAK1	ND	ND	-
JAK3	ND	ND	-
TYK2	ND	ND	-

The p-value was tested using the Mann–Whitney U test. Statistical significance was set at p-value < 0.05 was considered significant. SD, standard deviation; CXCL, C-X-C motif chemokine ligand; CXCR3; C-X-C motif chemokine receptor 3, IFN-γ, interferon γ; IL, interleukin; JAK, Janus kinase; TYK2, tyrosine kinase 2; ND, not detectable.

disease duration, or disease area (data not shown).

Some pro-inflammatory cytokines involved in the pathogenesis of AA (*2*); in particular, inhibition of JAK signaling has been attracting attention as a new strategy for treating AA, which acts to suppress inflammation (*6*). Serum protein levels of various cytokines such as serum C-C motif chemokine 17 /thymus and activation-regulated chemokine have potential as biomarkers of AA activity (*7*). Recently, elevated cfDNA levels have attracted attention for the potential of biomarkers in various diseases such as skin inflammatory diseases (*4,8*). For example, cfDNA levels in plasma are higher in patients with severe psoriasis vulgaris than those in healthy subjects (*4*). In autoimmune bullous diseases, serum cfDNA levels are increased compared with healthy volunteers. Especially, in patients with bullous pemphigoid (BP), serum cfDNA levels positively correlated with serum anti-BP180 antibody levels (*8*). Elevation of serum α1(I) collagen DNA levels in patients with systemic sclerosis might be useful as the diagnostic marker, reflecting the presence of vasculopathy (*9*). Phospholipase A2 group IV D (*PLA2G4D*) DNA copies in cfDNA in psoriatic patients are significantly higher than that in normal controls, and post-therapeutic circulating *PLA2G4D* DNA copies are significantly decreased after efficient therapy (*10*). We had conducted a comprehensive literature search by PubMed database, there was no literature about the correlation with cfDNA and AA. Thus, to the best of our knowledge, this is the first study to explore the significance of cfDNA levels in patients with AA. Furthermore, the expression of some circulating DNAs (*CXCL9*, *CXCL11*, *IL-15*, *IFN-γ*, and *JAK2*) was higher in patients with AA than in HC. These results suggest that increased circulating DNA levels may reflect damage to hair follicles in patients with AA.

However, these findings were based on a retrospective analysis and did not reveal the significant correlation between cfDNA levels and the clinical features of AA. Therefore, further extensive investigations are warranted to elucidate the clinical significance of cfDNA in patients with AA.

Funding: None.

Conflicts of Interest: The authors have no conflicts of interest to disclose.

References

1. Pratt CH, King LE Jr., Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. Nat Rev Dis Primers. 2017; 3:17011.
2. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: A review of disease pathogenesis. Br J Dermatol. 2018; 179:1033-1048.
3. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. DNA fragments in the blood plasma of cancer patients: Quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001; 61:1659-1665.
4. Beranek M, Fiala Z, Kremlacek J, Andrys C, Krejsek J, Hamakova K, Chmelarova M, Palicka V, Borska L. Changes in circulating cell free DNA and nucleosomes in patients with exacerbated psoriasis. Arch Dermatol Res. 2017; 309: 815-821.
5. Sakamoto R, Sawamura S, Kajihara I, Miyauchi H, Urata K, Otsuka-Maeda S, Kanemaru H, Kanazawa-Yamada S, Honda N, Makino K, Miyashita A, Aoi J, Makino T, Fukushima S, Ihn H. Circulating tumor necrosis factor-α DNA are elevated in psoriasis. J Dermatol. 2020; 47:1037-1040.
6. Lensing M, Jabbari A. An overview of JAK/STAT pathways and JAK inhibition in alopecia areata. Front Immunol. 2022; 13:955035.
7. Inui S, Noguchi F, Nakajima T, Itami S. Serum thymus and activation-regulated chemokine as disease activity and response biomarker in alopecia areata. J Dermatol. 2013; 40:881-885.
8. Kakitsuka Y, Sawamura S, Kajihara I, Kanemaru H, Honda N, Makino K, Aoi J, Makino T, Fukushima S, Ihn H.

Elevated circulating cell-free DNA levels in autoimmune bullous diseases. J Dermatol. 2020; 47:e345-e346.
9. Sawamura S, Jinnin M, Shimbara M, Nakamura K, Kudo H, Inoue K, Nakayama W, Kajihara I, Fukushima S, Ihn H. Serum levels of genomic DNA of α1(I) collagen are elevated in scleroderma patients. J Dermatol. 2017; 44:927-931.
10. Yamada-Kanazawa S, Mijiddorj MT, Kajihara I, Kanemaru H, Sawamura S, Makino K, Aoi J, Masuguchi S, Fukushima S. Circulating phospholipase A2 group IV D DNA copies are elevated in psoriasis. J Eur Acad Dermatol Venereol. 2022; 36: e302-e304.

Received April 1, 2024; Revised April 5, 2024; Accepted April 11, 2024.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Ikko Kajihara, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan.
E-mail: kajiderma@gmail.com

Released online in J-STAGE as advance publication April 13, 2024.