Received: 29 September 2020 | Revised: 15 October 2020 | Accepted: 21 October 2020

DOI: 10.1111/jocd.13814

ORIGINAL CONTRIBUTIONS

JCD Journal of Cosmetic Dermatology WILEY

Cross talk between oxidative stress and inflammation in alopecia areata

Amany Ibrahim Mustafa MD[1] | Rana Atef Khashaba MD[2] | Eman Fawzy PhD[3] | Shimaa Mohamad AbdElRahman Baghdady MD[4] | Shymaa Mostafa Rezk MD[1]

[1]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Benha University, Benha, Egypt

[2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Benha University, Benha, Egypt

[3]Department of Laboratory Medicine, Mansoura Fever Hospital, Ministry of Health, Mansoura, Egypt

[4]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Benha University, Benha, Egypt

Correspondence
Amany Ibrahim Mustafa, Department of Dermatology, Venereology andAndrology, Faculty of Medicine, Benha University, Benha, Egypt.
Email: amanyIbrahim26@yahoo.com

Abstract

Background: Although the etiopathogenesis of alopecia areata (AA) is still unclear, inflammation, oxidative stress, and subsequent DNA damage might be considered role players in disease development.

Aim: We aimed at exploring the potential link between oxidative DNA damage and inflammation in AA patients through measuring 8-hydroxy deoxyguanosine (8-OHdG), high mobility group box 1 protein (HMGB1), and one of the inflammatory mediators, C-reactive protein (CRP).

Methods: A total of 79 subjects (49 AA patients in addition to 30 apparently healthy control subjects) were tested for serum levels of 8-OHdG, HMBG1, and CRP.

Results: Compared with the control group, serum 8-OHdG, HMBG1, and CRP levels were significantly elevated in the studied patients group (0.031, <0.001, and <0.001, respectively). Moreover, logistic regression analysis revealed that disease course, serum levels of 8-OHdG, and HMBG1 were considered independent predictors for AA severity in both uni- and multivariable analyses.

Conclusion: Our results suggest a possible role of oxidative stress together with pro-inflammatory biomarkers in development of AA and their benefit in predicting a severe form of the disease.

KEYWORDS

8-OHdG, Alopecia areata, CRP, HMGB1, Oxidative stress

1 | INTRODUCTION

Alopecia areata (AA) is the most frequent, localized, nonscarring hair loss that affects any hair bearing skin.[1] The lifetime risk of AA in the general population is about 2%.[2] It most commonly presents by single or multiple distinct, well-defined round or oval patches of hair loss on the scalp or body, the disease might involve the whole scalp (alopecia totalis) or the total body (alopecia universalis).[3] Alopecia areata is a complex disease where many factors assumed to be involved in its development, such as autoimmunity, genetic constitution, and emotional and environmental stress.[4] Cells infiltrating the skin after immunological and/or nonimmunological

stimuli; release various bioactive products as inflammatory cytokines and reactive oxygen species (ROS) that may denature proteins, change apoptosis, and affect the inflammatory mediators release leading to the development of cutaneous tumors, skin aging and inflammatory cutaneous disorders.[5] 8-hydroxy deoxyguanosine (8-OHdG) and high mobility group box 1 protein (HMGB1) exist at the sites of oxidative DNA damage in living cells and act as sensitive markers for it.[6,7] The acute-phase reactant, C-reactive protein (CRP), is diagnostic biomarker for inflammation in different diseases.[8]

Thus, to evaluate AA-associated oxidative DNA damage and explore the potential link between oxidative stress and inflammatory response in AA, 8-hydroxy deoxyguanosine (8-OHdG), high mobility group box 1 protein (HMGB1), and C-reactive protein (CRP) were investigated among AA patients.

2 | SUBJECTS AND METHODS

2.1 | Study design

This study included 49 patients with alopecia areata (AA) who referred to our dermatology clinic from April 2018 to 2019 and 30 sex- and age-matched healthy persons. The study had been assessed and agreed by the institutional ethical committee according to Helsinki Declaration principles. Studied subjects received comprehensive information about the current research and filled a written informed consent prior to participation in the study.

2.2 | Study Population

Diagnosis of AA was clinically based on the presence of well-defined patches of hair loss in the scalp or large hairless areas in normal-appearing skin. The studied patients suffering from AA who are either not treated before or stopped treatment for minimally two months. Pregnant or lactating females, patients below 12 years old, suffering from anemia, bleeding disorders, other autoimmune diseases, systemic inflammatory diseases, or inflammatory skin disorders were not included in the present work.[9]

2.3 | Methods

For evaluating the relation of different clinical characteristics with serum markers, we assessed patients' essential information and all patients were subjected to complete history taking and general examination according to recommended investigative guidelines for AA [10] including age, sex, course, duration, family history, pattern, extent of alopecia and severity of disease, and concomitant nail pitting. The scalp was examined to determine configuration, number, and size of AA patches. Severity of the disease was scored according to the "Severity of Alopecia Tool" (SALT) score defined by Olsen. [10]

2.4 | Laboratory investigations

Three ml venous blood was collected from each subject by clean venipuncture. Serum was obtained by centrifugation of clotted samples at 1000 *g* for 15 minutes. All samples were coded and stored at −20°C. Two different sandwich ELISA kits for research use only were used to detect the serum levels of 8-OHdG (Cat #: K4160, BioVision Inc, Milpitas, California, USA) and HMGB-1 (Cat #: 11 683, Glory Science Co). The serum level of CRP was detected using a diagnostic quantitative latex agglutination assay (Cat #: 13 921, Biosystems, Barcelona, Spain) by the turbidimetry method at wavelength 540 nm. The reference serum adult level of CRP is up to 5 mg/L.

2.5 | Statistical methods

For continuous variables, values were reported as mean ± standard deviation (SD) or median (interquartile range). In categorical variables, data were expressed as the number of subjects (percentage). To compare the continuous variables, the nonparametric the Mann-Whitney *U* test was applied and Kruskal-Wallis test was carried out wherever appropriate. Logistic regression was used to predict disease severity among studied patient. Statistical analysis was performed using the statistical software SPSS 16.0.0. (SPSS Inc Chicago). *P* values <.05 were considered statistically significant.

3 | RESULTS

3.1 | Sociodemographic characteristics of the studied subjects

This study included 49 patients with alopecia areata (31 males and 18 females) with a mean age of 31. 9 ± 10.1 years, in addition to 30 healthy control subjects (14 males and 16 females). The mean age of the control subjects was 35.8 ± 11.4 years. The basic sociodemographic and clinical features of the studied subjects are conferred in Table 1.

3.2 | Oxidative DNA damage and inflammatory biomarkers

Serum 8-OHdG, HMBG1, and CRP levels were showing significant higher levels in patients group than control subjects (P 0.031, < 0.001 and < 0.001, respectively) (Table 1).

3.3 | Relation between serum biomarkers and clinical parameters in studied AA patients

Comparing the serum levels of oxidative DNA damage and inflammatory biomarkers regarding the clinical parameters in the studied

TABLE 1 Sociodemographic characteristic and baseline data of the studied subjects

		Patients (N = 49)	Control (N = 30)	*P*
Age (years) mean ± SD		31.9 ± 10.1	35.8 ± 11.4	.121
Gender (M/F) N		31/18	14/16	.148
Duration (years) median (range)		1 (0.25-4)	-	-
Course N (%)	Constant	26 (53.1%)	-	-
	Progressive	23 (46.9%)	-	-
Pattern N (%)	Patchy	34 (69.4%)	-	-
	Totalis	10 (20.4%)	-	-
	Ophiasis	3 (6.1%)	-	-
	Universalis	2 (4.1%)	-	-
SALT score N (%)	S1	21 (42.9%)	-	-
	S2	6 (12.2%)	-	-
	S3	5 (10.2%)	-	-
	S4	5 (10.2%)	-	-
	S5	12 (24.5%)	-	-
Family history N (%)	Positive	8 (16.3%)	-	-
	Negative	41 (83.7%)	-	-
8-OHdG (ng/ml) median (range)		20.8 (4.3-95.1)	16.2 (5.5-58.6)	**.031***
HMGB1 (ng/ml) median (range)		49.1 (10-74)	3.3 (0.3-15)	**<.001***
CRP (mg/L) median (range)		5.2 (0.5-32)	1.9 (0.1-5.0)	**<.001***

Abbreviations: SALT, Severity of Alopecia Tool; SD, standard deviation.

patients reveled that their levels were significantly higher among AA patients with extensive disease patterns ($P < .001$) and those with higher SALT score ($P = .008$) being the highest in patients with alopecia totalis and SALT score S5 (Table 2).

3.4 | Performance characteristics of serum biomarkers for discrimination between AA patients and control groups

Receiver operating curve of serum levels of CRP, HMGB1, and 8 OHdG was conducted for discrimination between AA patients and control groups. HMGB1 showed excellent AUC, CRP showed good AUC, whereas 8 OHdG showed poor AUCs. Other performance characteristics are shown in Table 3 and Figure 1.

3.5 | Prediction of AA severity in the studied patients group

Regression analysis was conducted for prediction of severity among AA patients, using age, gender, disease duration, disease course and serum levels of 8-OHdG, HMGB1 and CRP as covariates. Progressive disease course, serum levels of the tested biomarkers were considered as independent predictors for increasing disease severity in both uni- and multivariable regression analyses (Table 4).

4 | DISCUSSION

Alopecia areata is a common disorder with inflammation-mediated hair loss. Its etiopathogenesis and underlying mechanisms are unclear, although different theories were suggested including infections and neurological, genetic, and autoimmune hypotheses.[11] Oxidative stress is a probable mechanism for AA with an increase in the free radicals production exceeding the intracellular antioxidant defense that results in damaging of cellular components with release of various products that might be used as biomarkers for disease development and severity.[4]

Among the released bioactive products, the 8-Hydroxy deoxyguanosine (8-OHdG) is the oxidative modification of the DNA guanosine nitrogen base, and it can act as a precise biomarker for oxidative DNA damage.[6] Also, the high mobility group box 1 protein (HMGB1) was initially recognized as one of the DNA-binding proteins and also identified as damage-associated molecular pattern (DAMP).[12] HMGB1 accumulation into the extracellular matrix exists

TABLE 2 Relationship between serum levels of 8-OHdG, HMGB1, and CRP and clinical parameters in studied AA patients

		AA patients N = 49								
		8-OHdG (ng/mL) median (range)			HMGB1 (ng/mL) median (range)			CRP (mg/L) median (range)		
		Median	Range		Median	Range		Median	Range	
Course	Constant	18.6	4.3	35.8	48	10	62.8	3.1	0.5	15
	Progressive	32.3	12.7	95.1	53	32.1	74	13.6	0.6	32
	P	**<.001**[a]			**.013**[a]			**.003**[a]		
Pattern	Patchy	19.5	4.3	79.5	46.8	10	74	3.1	0.5	32
	Totalis	62.2	21.3	95.1	68.1	53	73.5	20	5.9	32
	Ophiasis	16.9	13.5	42.0	49.1	45.7	63	5.2	1.3	13.6
	Universalis	13.2	10.6	15.8	40.1	32.1	48	3.1	2.3	3.9
	P	**.001**[a]			**<.001**[a]			**.003**[a]		
SALT score	S1	13.6	4.3	62.1	36.8	10	74	2.2	0.5	32
	S2	19.2	13.5	31.7	48.5	48	51.3	3.7	1.4	15
	S3	32.3	21.9	37.5	51.3	51.3	53	14	11.4	15
	S4	20.8	17.7	26.8	54.5	53	61.5	10.8	2.1	12
	S5	74.6	21.3	95.1	66.4	61.5	73.5	20	4.1	32
	P	**<.001**[a]			**<.001**[a]			**<.001**[a]		
Family history	Positive	20.5	4.3	89.3	49.1	10	74	4.1	0.5	32
	Negative	24.1	10.6	95.1	51.2	24.5	72.5	8.5	0.5	24
	P	0.607			0.914			0.695		
Nail pitting	Positive	20.6	4.3	89.3	48.5	10	74	4	0.5	32
	Negative	32.3	11.5	95.1	49.1	24.5	72.5	6.2	0.5	24
	P	.296			.948			.561		

Abbreviation: SALT, Severity of Alopecia Tool.

[a]Bold indicate significant values.

TABLE 3 AUC and performance characteristics of HMBG and OHdG for discrimination between AA cases and control groups

	CRP (mg/L)	HMGB1 (ng/ml)	8-OHdG (ng/ml)
AUC	0.773	0.998	0.693
95% CI	0.673-0.873	0.992-1	0.479-0.730
P	**<.001**[a]	**<.001**[a]	**.045**[a]
Cutoff value	5.1	16	18.7
Sensitivity (%)	51	95.9	61.2
Specificity (%)	100	100	63.3
PPV (%)	100	100	73.2
NPV (%)	55.6	93.8	50
Accuracy (%)	69.6	97.5	62

Abbreviations: AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

[a]Bold indicate significant values.

in sites of oxidative DNA damage in viable cells, so it is defined as a constituent of the primary DNA damage response,[13] while the elevated level of C-reactive protein (CRP), a crucial biological marker of acute-phase systemic inflammatory response, can predict inflammation in different diseases.[8] In the current study, we aimed at evaluation of oxidative stress associated with AA via the measurement of serum 8-OHdG and HMGB1 levels besides serum CRP level as an inflammatory marker and their possible role in disease pathogenesis and severity.

The current study results revealed a significant elevation in serum 8-OHdG level in AA patients than healthy control subjects. Moreover, 8-OHdG was found to be an independent predictor for disease severity in AA patients. However, it did not differ with other clinical characteristic in studied patients. Oxidative stress occurs when the natural oxidant-antioxidant balance disturbed when there is an overproduction of free radicals or un-functioning antioxidant defense. The skin is continuously exposed to either intrinsic or extrinsic environmental oxidative forces resulting in ROS production, with subsequent damage of the cellular components such as nucleic acids, proteins, and cell membrane lipids.[5] Oxidative stress has been involved in several dermatopathologies as psoriasis,[14] vitiligo,[15] atopic dermatitis, lichen planus,[16] acne vulgaris,[17] pemphigus vulgaris,[18] seborrheic dermatitis,[19] and skin cancers.[20] Oxidative stress role in AA pathogenesis is controversial, as it was found that total

antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) did not significantly differ between AA patients when compared to controls.,[21] while others observed a significant lower TAS with increased free radical levels in AA patients than controls. Thus, oxidative DNA damage in terms of modified nitrogenous bases may carry mutations to the subsequent generations of the hair follicles, making them more vulnerable to apoptosis and the inflammatory responses observed in AA patients and might worsen disease status. [22,23]

Regarding HMGB1, our study results revealed that its serum level was not only significantly higher in AA patients compared to control subjects but also its level showed a significant elevation in patients with extensive disease patterns and higher SALT score grades. Also, it was found to be an independent predictor for disease severity in AA patients as well. HMGB1 had been studied by Lee et al[24] who found that its level was significantly elevated in the studied AA patients than control subjects and in alopecia totalis patients than those with other disease patterns. HMGB1 is produced mainly by necrotic cells in response to variable stimuli. It mediates cellular responses as chemotactic cell movement and inflammatory cytokine (TNF-α, IL-1*b*, and IL-6) release, and affects various immune cells (macrophages, monocytes, T and B cells).[25] Also, it is supposed to be an essential target antigen in some immune-mediated disorders.[24] Thus, our results support the theory of HMGB1 implication in AA pathogenesis and reflecting disease severity. The initial increase in HMGB1 levels may lead to the destruction of immune privilege of hair follicles thus initiating the autoimmunity in AA. Also, the secretion of HMGB1 and subsequent proinflammatory cytokines occurs during AA may lead to propagation of inflammation results in more severe disease. The current work results also revealed, as expected, that the CRP was significantly higher in AA patients than healthy control subjects what comes in line with Mahamid et al [26] who revealed a mild but insignificant elevation of CRP levels in AA patients supporting the role of the inflammatory mechanism in disease development. However, Yousefi et al [27] when assessing serum hsCRP levels in AA patients and healthy control subjects did not find significant difference. CRP is a member of the pentraxin family of oligomeric proteins that are implicated in the innate immune response. Its immune regulatory functions include amplification of leukocytic activity, complement fixation, modulation of platelet activation, and clearance of cellular debris from sites of active inflammation.[28] Thus, it could be considered as a biomarker of systemic inflammatory response promoting the secretion of other inflammatory mediators.[29]

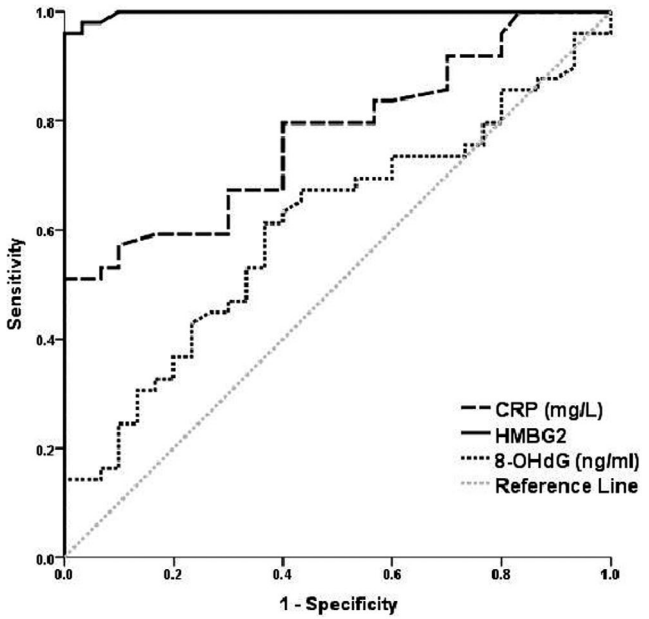

FIGURE 1 ROC curve of serum CRP, HMBG, and 8-OHdG levels for discrimination between AA patients and control groups

5 | CONCLUSION

Our findings support the role of oxidative stress in AA and suggest a potential link between oxidative stress-induced DNA damage markers (8-OHdG & HMGB1) and proinflammatory markers (CRP), with the disease pathogenesis and severity. Modulating the activity of these biomarkers might provide a new perspective for treating patients with AA in order to improve their quality of life.

TABLE 4 Regression analysis for prediction of AA severity among the studied patients

	Univariable regression analyses		Multivariable regression analyses	
	P	OR [95% CI]	*P*	OR [95% CI]
Age	.083	1.027 [0.997-1.058]		
Gender	.379	0.763 [0.418-1.394]		
Duration	.296	1.171 [0.871-1.574		
Progressive course	**.035**[a]	**1.583 [1.875-6.846]**	0.192	1.439 [0.729-2.198]
Serum 8-OHdG	**.001**[a]	**1.297 [1.085-2.009**	**.023**[a]	**1.329 [1.128-2.176]**
Serum HMGB1	**.008**[a]	**1.030 [1.008-1.052]**	**<.001**[a]	**1.043 [1.019-1.067**
Serum CRP	**.005**[a]	**1.696 [1.164-1.928]**	**.002**[a]	**1.265 [1.028-1.820]**

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Bold indicate significant values.

ACKNOWLEDGMENTS

We are extremely grateful to all volunteers who took part in this study and the research team who collected the data.

CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

Mustafa AI MD and Rezk SM MD performed the research. Mustafa AI MD designed the research study. Khashaba RA MD and Abd El Rahman SM MD contributed essential reagents or tools. Fawzy E phD analyzed the data. Mustafa AI MD, Rezk SM MD, and Fawzy E phD wrote the paper.

ETHICAL APPROVAL

The study was approved by the local ethics committee on research involving human subjects in the faculty of Medicine; Benha University in agreement with the Declaration of Helsinki. An informed consent was obtained from each subject prior to participation.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

ORCID

Amany Ibrahim Mustafa https://orcid.org/0000-0003-3877-5787

REFERENCES

1. Bakry OA, Basha MA, El Shafiee MK, Shehata WA. Thyroid disorders associated with alopecia areata in egyptian patients. *Indian J Dermatol*. 2014;59(1):49-55.
2. Villasante Fricke AC, Miteva M. Epidemiology and burden of alopecia areata: a systematic review. *Clin Cosmetic Invest Dermatol*. 2015;8:397-403.
3. Hordinsky MK. Overview of alopecia areata. *J Investig Dermatol Symp Proc*. 2013;16(1):S13-S15.
4. Prie B, Voiculescu V, Ionescu-Bozdog O, et al. Oxidative stress and alopecia areata. *J Med Life*. 2015;8:43-46.
5. Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. *J Invest Dermatol*. 2006;126:2565-2575.
6. Helbock HJ, Beckman KB, Ames BN. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine as biomarkers of oxidative DNA damage. *Methods Enzymol*. 1999;300:156-166.
7. Prasad R, Liu Y, Deterding LJ, et al. HMGB1 is a cofactor in mammalian base excision repair. *Mol Cell*. 2007;27:829-841.
8. Borska L, Kremlacek J, Andrys C, et al. Systemic Inflammation, Oxidative Damage to Nucleic Acids, and Metabolic Syndrome in the Pathogenesis of Psoriasis. *Int. J Mol Sci*. 2017;18:2238.
9. Magna M. Pisetsky DS (2014) The Role of HMGB1 in the Pathogenesis of Inflammatory and Autoimmune Diseases. *Mol Med*. 2014;20(1):138.
10. Olsen EA. Investigative guidelines for alopecia areata. *Dermatol Ther*. 2011;24:311-319.
11. Gonul M, Cakmak SK, Soylu S, et al. Serum vitamin B12, folate, ferritin, and iron levels in Turkish patients with alopecia areata. *Indian J Dermatol Venereol Leprol*. 2009;75:552.
12. Said-Saidier N. Ojcius M (2012): Alarmins, inflammasomes and immunity. *Biomed J*. 2012;35:437.
13. Tang D, Kang R, Zeh HJ, Lotze MT. High-mobility group box 1, oxidative stress, and disease. *Antioxid Redox Signal*. 2011;14(7):1315-1335.
14. Bacchetti T, Campanati A, Ferretti G, et al. Oxidative stress and psoriasis: the effect of antitumour necrosis factor-α inhibitor treatment. *Br J Dermatol*. 2013;168(5):984-989.
15. Akoglu G, Emre S, Metin A, et al. Evaluation of total oxidant and antioxidant status in localized and generalized vitiligo. *Clin Exp Dermatol*. 2013;38(7):701-706.
16. Sapuntsova SG, Lebed'ko OA, Shchetkina MV, et al. Status of free-radical oxidation and proliferation processes in patients with atopic dermatitis and lichen planus. *Bull Exp Biol Med*. 2011;150(6):690-692.
17. Grange PA, Weill B, Dupin N, Batteux F. Does inflammatory acne result from imbalance in the keratinocyte innate immune response? *Microbes Infect*. 2010;12(14–15):1085-1090.
18. Yesilova Y, Ucmak D, Selek S, et al. Oxidative stress index may play a key role in patients with pemphigus vulgaris. *J Eur Acad Dermatol Venereol*. 2013;27(4):465-467.
19. Ozturk P, Arican O, Kurutas EB, et al. Oxidative stress in patients with scalp seborrheic dermatitis. *Acta Dermatovenerol Croat*. 2013;21(2):80-85.
20. Sander CS, Hamm F, Elsner P, Thiele JJ. Oxidative stress in malignant melanoma and non-melanoma skin cancer. *Br J Dermatol*. 2003;148(5):913-922.
21. Motor S, Ozturk S, Ozcan O, et al. Evaluation of total antioxidant status, total oxidant status and oxidative stress index in patients with alopecia areata. *Int J Clin Exp Med*. 2014;7(4):1089-1093.
22. Bilgili SG, Ozkol H, Karadag AS, et al. Serum paraoxonase activity and oxidative status in subjects with alopecia areata. *Cutan Ocul Toxicol*. 2013;32:290-293.
23. Kim SW, Kim BJ, Youn SW, et al. Evaluation of free oxygen radical and antioxidant capacity in alopecia areata. *J Dermatol*. 2010;37:762-764.
24. Lee Y, Lee HE, Shin JM, et al. (2013) Clinical significance of serum high-mobility group box 1 level in alopecia areata. *J Am Acad Dermatol*. 2013;69:742-747.
25. Yang H, Tracey KJ. Targeting HMGB1 in inflammation. *Biochim Biophys Acta*. 2010;1799:149-156.
26. Mahamid M, Abu-Elhija O, Samamra M, et al. Association between Vitamin D levels and Alopecia Areata. *Isr Med Assoc J*. 2014;16(6):367-370.
27. Yousefi M, Namazi MR, Rahimi H, et al. Evaluation of Serum Homocysteine, High-Sensitivity CRP, and RBC Folate in Patients with Alopecia Areata. *Indian J Dermatol*. 2014;59(6):630.
28. Pradhan AD, Manson JE, Rifai N, et al. C-Reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286:327-334.
29. Shamsuzzaman AS, Winnicki M, Lanfranchi P, et al. Elevated C-reactive protein in patients with obstructive sleep apnea. *Circulation*. 2002;105:2462-2464.

How to cite this article: Mustafa AI, Khashaba RA, Fawzy E, Baghdady SMA, Rezk SM. Cross talk between oxidative stress and inflammation in alopecia areata. *J Cosmet Dermatol*. 2021;20:2305–2310. https://doi.org/10.1111/jocd.13814