Archives of Dermatological Research (2019) 311:589–594
https://doi.org/10.1007/s00403-019-01934-9

ORIGINAL PAPER

Macrophage migration inhibitory factor polymorphism (rs755622) in alopecia areata: a possible role in disease prevention

Fateme Rajabi[1,3] · Mahsa M. Amoli[2] · Reza M. Robati[1] · Mina Almasi-nasrabadi[1] · Navid Jabalameli[3]

Received: 11 March 2019 / Accepted: 24 May 2019 / Published online: 1 June 2019

Abstract

Alopecia areata (AA) is an organ-specific autoimmune disease that targets the bulb of the hair follicles and results in non-scarring hair loss that can range from patchy lesions to involvement of the entire scalp. AA develops when the hair follicles lose their physiologic state of immune privilege. One of the key factors that help in maintaining this immune privilege by suppressing natural killer cells is macrophage migration inhibitory factor (MIF). Surprisingly, MIF is also known to provoke autoimmunity by upregulating cytokines. To address this dilemma and understand the exact nature of the involvement of MIF in disease pathogenesis we investigated the association of MIF gene polymorphisms (− 173 G > C, rs755622) with AA by conducting a case–control study of 274 subjects. We observed that the frequency of the C allele in the patients was significantly lower than the control group (0.15, 0.23, respectively, $p = 0.01$) and the combined frequencies of the CC and GC genotypes (dominant Mendelian pattern) had the most prevalent difference between the two groups (odds ratio 0.60, 95% confidence interval 0.36–0.99; $p = 0.048$).Since the C allele is associated with higher MIF transcription levels, this could infer that MIF is more likely to attribute to the preservation of the immune privilege rather than acting as a proinflammatory factor.

Keywords Immune privilege · Autoimmunity · Natural killer cells · Genetic association study · Hair follicle

Introduction

Alopecia areata (AA) is a general term describing a non-scarring inflammatory hair loss with a wide range of manifestations from involving patchy areas in the scalp to causing hair loss in the entire scalp and body (alopecia totalis and universalis, respectively). The disease has devastating psychological impacts and no effective treatment to date. AA can affect up to two percent of the population [19].

The pathogenesis of AA is thought to be due to the loss of the immune privilege of anagen follicles. In a physiological state, the hair follicles remain out of reach of the immune system via reduced expression of major histocompatibility complexes (MHCs) that prevent T-cell recognition [9, 12]. Since natural killer cell (NK cells) are capable of detecting and destroying MHCs negative cells, hair follicles have adopted few strategies to actively suppress them and maintain the immune privilege. One of these mechanisms is the production of macrophage migration inhibitory factor (MIF) [14, 18].

MIF is a 12.5 kDa protein with a wide range of functions (ranging from an immune regulator to a catalytic enzyme). In many immune-privileged sites such as the eye, hair follicles, and nails, MIF is thought to possess an essential anti-inflammatory role by suppressing NK cells and preserving the immune privilege [1, 13, 26]. However, MIF has also been described as a counter-regulatory factor induced by glucocorticoids in a state of local or systemic stress [7] that could promote the innate and adaptive immune responses by upregulating proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), IL-6, IL-8, IL-12, IL-17, and interferon gamma (IFNγ) [3].

The various proinflammatory functions of MIF are in contrary to its presumed roles in upholding the immune privilege. The significantly higher levels of MIF in many

✉ Reza M. Robati
rezarobati@sbmu.ac.ir

1 Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Metabolic Disorders Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

3 Network of Dermatology Research (NDR), Universal Scientific Education and Research Network (USERN), Tehran, Iran

autoimmune diseases cause additional controversy in this matter [8, 21, 23, 35, 36].

We believe that higher tissue and serum levels of MIF should not be interpreted as the source of inflammation; nevertheless, it could be a consequence of the inflammation leading to elevated glucocorticoid levels that could induce MIF expression. With this theory in mind, we sought to address this dilemma from a genetic point of view.

A single nucleotide polymorphism (SNP) in the 5-flanking region of the promoter of the MIF gene (− 173 G > C, rs755622) is known to have a significant effect on transcription levels.

This SNP is the most widely studied polymorphism of the MIF gene and many autoimmune diseases have been linked to it [11]. Our aim was to investigate whether AA imitates other autoimmune diseases in the association with MIF (− 173 G > C) gene variants.

Materials and methods

Subjects

We conducted a case–control study with the patients and the control subjects recruited from individuals referred to our dermatology clinics. The Ethics Committee approved this study and written informed consent was obtained from all participants according to the declaration of Helsinki.

The study consisted of 152 healthy volunteers and 122 patients diagnosed with AA based on clinical, dermoscopy, and histopathological evaluations. The participants were consecutively recruited over a period of 114 months (2017–2018). None of the participants were first degree relatives.

The control group consisted of individuals referred to our clinics for cosmetic concerns. We excluded those with personal or family history of AA or any other autoimmune disease from the control group.

We documented demographic data for each individual. The disease characteristics such as age at onset, involvement of nails, body hair involvement, and disease severity, presence of an accompanying autoimmune disease, and family history of AA were also documented in the patient group. The severity of hair loss was assessed by a trained dermatologist according to the severity of alopecia tool score (SALT score) [24].

Genotyping

Blood samples were collected in 5 mL ethylenediaminetetraacetic acid tubes. DNA was isolated using the salting-out method. MIF genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. This method has been previously described in detail [5, 27, 29]. Briefly, the PCR mixture was composed of a total reaction volume of 25 mL with the following elements: DNA, 200 ng; primers (forward sequence 5′-ACT-AAG-AAA-GAC-CCGAGGC-3′ and reverse sequence 5′-GGG-GCA-CGT-TGG-TGT-TTA-C-3′), 0.3 mM of each; dNTPs, 0.2 mM; taq polymerase (Fermentas), 1 unit; 2 mL 10 × PCR buffer (Fermentas); magnesium chloride 2.5 mM.

The cycling settings for the PCR mixture was as follows: 95 °C for 10 min, 35 amplification cycles at 95 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s and then an extension at 72 °C for 10 min.

The PCR products were then incubated overnight at 37 °C and digested using AluI restriction endonuclease (Fermentas). The products were visualized by 3% agarose gel electrophoresis stained with SYBR green. The PCR products for the GG genotype had two fragments (98 and 268-bp), the CC genotype had three fragments (205, 98 and 63-bp) and the GC genotype had four fragments (268, 205, 98 and 63-bp). The gel was then placed on a UV trans-illuminator for photographing purpose.

Statistical analysis

Statistical analysis was performed by SPSS statistical software package (version 24.0). Alpha level of 0.05 and power of 80% was used to determine the sample size. Hardy–Weinberg equilibrium was checked using the Chi-square (χ^2) test. Allele and genotype frequencies for MIF gene polymorphisms were determined. Chi-square (χ^2) test and Fisher's exact test were used to compare allele and genotype frequencies between the two groups. The odds ratio (OR) and 95% confidence intervals (CI) were measured for each allele and genotypes. A p values of less than 0.05 were considered significant. Alpha level of 0.05 and power of 80% was used to determine the sample size.

Results

The study consisted of 122 patients diagnosed with alopecia areata and 152 control subjects. The demographic information and basic disease characteristics are demonstrated in Table 1. All the individuals enrolled in this study were from the same population. The genotype frequencies of both groups were in Hardy–Weinberg equilibrium ($p = 0.90$). We were not able to match the case–control subjects according to gender (35.2% females in the patient group and 52% in the control group, $p = 0.03$) but the two groups did not differ significantly in age (27.03 ± 10.37 years in the patients with AA vs. 29.45 ± 11.27 years in the controls, $p = 0.71$).

Table 1 The demographic information and basic disease characteristics

Characteristics	Alopecia areata	Controls
Female	43 (35.2%)	73 (52%)
Male	79 (64.8%)	79 (48%)
Age (year)	27.03 (± 10.37)	29.45 (± 11.27)
Age at onset (year)	25.22 (± 12.06) Range 3–70	–
SALT score[a]		–
Score1	45 (38.5%)	
Score2	10 (8.5%)	
Score3	12 (10.3%)	
Score4	50 (42.7%)	
Thyroid disease	22 (18.6%)	–
Nail involvement	69 (58.5%)	–
Body hair involvement	70 (59.3%)	–

[a]Severity of alopecia tool score, 1 (less than 25% scalp hair involvement), 2 (25–49%), 3 (50–74%), and 4 (75–100%)

The frequency of the C allele in the patients was significantly lower than the control group (0.15, 0.23, respectively, $p = 0.01$). The two groups also differed significantly in the frequency of their genotypes ($p = 0.038$). Since the combined frequencies of the CC and GC genotypes, as opposed to the GG genotype, had the most prevalent difference between the patients and controls, a dominant Mendelian pattern best corresponded with the genotype distribution. This indicated that individuals with CC and GC genotypes had a higher chance of being protected against disease development (Table 2). However, AA is a polygenic disease and it is not of our direct interest to look for an inheritance pattern.

We also performed subgroup analysis by stratifying the patients according to sex, age at onset (according to the median that was 25), disease severity (according to SALT score), and the presence of thyroiditis and nail involvement. The allelic and genotype frequencies did not differ significantly within the subgroups except for the presence of thyroiditis. The distribution of genotypes in patients with thyroiditis seemed to have a significant difference with those without thyroiditis but with a marginal p value (Table 3).

Discussion

In the present study, we were able to demonstrate a lower expression of the C allele in individuals with AA. Considering the higher transcription levels associated with the C allele, that is thought to be due to the formation of an activator protein-4 response element [4, 17, 20], we believe that the proinflammatory roles of MIF might not be as essential to the inflammatory cascades in AA as its immune preservation roles are to the immune privilege of the hair follicles.

At least several studies have been conducted investigating the links between MIF and AA.

Regarding the MIF gene, the locus containing this gene (22q11) did not show an association with AA in a large scale GWAS study [25]. However, it was still essential to conduct an association study for these polymorphisms since GWAS studies usually do not detect rare allele variants or large effect sizes. Moreover, an association study by Shimizu et al. has been able to demonstrate a significant association between the MIF gene polymorphisms and AA in the Japanese population [32]. In the mentioned study two genetic polymorphisms of the promoter region (− 173 G < C and − 794 [CATT] $_{5–8}$ repeat) were compared between 113 patients with extensive alopecia and 194 healthy controls. In contrary to our study, they found that carriers of the C allele (GC and CC genotypes) had a higher risk of developing early onset severe AA [32]. The discrepancies between our study and the findings in the study by Shimizu et al. might be explained by the different ethnic origins and the differences in the degree of hair loss in the individuals enrolled in each study (our study includes patients with a milder disease).

The MIF gene (precisely the – 173 G > C or rs755622 SNP) has also been studied for many autoimmune and inflammatory diseases and in most of these cases, the CC genotype has shown a strong effect on disease development.

As for histological studies regarding MIF in AA, the findings are contradictory. Shimizu et al. performed a histological investigation that revealed intensive MIF staining in samples from AA lesions as compared to healthy controls [33]. In a study by Kang et al. quantitative PCR was employed to elucidate MIF expression. They compared four tissue

Table 2 The frequencies of alleles and genotypes in each group

MIF – 173 G/C	Control subjects	Alopecia areata	1/Odds ratio (95% CI)	p value*	χ^2
C	69 (23%)	36 (15%)	1.69 (1.08–2.64)	0.01	5.51
G	235 (77%)	208 (85%)			
CC	8 (5.2%)	1 (0.8%)	0.60 (0.36–0.99)[a]	0.048[a]	3.89[a]
GG	91 (59.9%)	87 (71.3%)			
GC	53 (34.9%)	34 (27.9%)			

*A p value of less than 0.05 was considered significant

[a]Comparing the CC + GC genotypes to the GG genotype via Chi-square test

Table 3 The distribution of alleles and genotypes among subgroups of alopecia areata

MIF – 173 G/C[a]	C	G	*p* value	CC	GG	GC	*p* value	*p* value for CC + GC vs. CC
SALT score[b]			0.71				0.60	0.55
Score1	16 (16%)	84 (84%)		0	33 (67.3%)	16 (32.7%)		
Score2	4 (20%)	16 (80%)		0	6 (60%)	4 (40%)		
Score3	2 (8.3%)	22 (91.7%)		0	10 (83.3%)	2 (16.7%)		
Score4	14 (14%)	86 (86%)		1 (2%)	38 (74.5%)	12 (23.5%)		
Thyroiditis			0.064				0.047	0.10
Present	5 (11.4%)	39 (88.6%)		18 (81.8%)	3 (13.6%)	1 (4.6%)		
Absent	31(15.5%)	169 (84.5%)		69 (69%)	31 (31%)	0		
Nail involvement			0.85				1.0	0.93
Present	21 (15.2%)	117 (84.8%)		1 (1.5%)	49 (71%)	19 (27.5%)		
Absent	15 (14.2%)	91 (85.8%)		0	38 (71.7%)	15 (28.3%)		
Body hair loss			0.81				0.81	0.66
Present	20 (14.3%)	120 (85.7%)		1 (1.4%)	51 (72.9%)	18 (25.7%)		
Absent	16 (15.4%)	88 (84.6%)		0	36 (69.2%)	16 (30.8%)		
Age at onset			0.59				0.76	0.66
< 25	19 (16.1%)	99 (83.9%)		1 (1.7%)	41 (69.5%)	17 (28.8%)		
> 25	17 (13.5%)	109 (86.5%)		0	46 (73%)	17 (27%)		

[a]The Chi-square test was utilized for all comparisons. A *p* value of less than 0.05 was considered significant

[b]Severity of alopecia tool score, 1 (less than 25% scalp hair involvement), 2 (25–49%), 3 (50–74%), and 4 (75–100%)

samples from individuals with AA (both lesional and perilesional) with four samples from normal control subjects. They were able to demonstrate higher expression of the MIF gene in alopecic lesions compared to control subjects, but the difference was not statistically significant [15]. On the contrary, in an immunohistological study by Ito et al. MIF showed a significantly higher expression in normal hair follicles compared to alopecic lesions (they compared eight AA samples with five healthy controls) [14]. Furthermore, three studies have been able to detect higher serum levels of MIF in patients diagnosed with AA. In these studies, MIF levels correlated with disease severity, disease duration, earlier age of onset, and presence of psychological stress [30, 33, 38].

MIF is generally increased in the skin in inflammatory conditions such as atopic dermatitis [31], psoriasis [34], systemic lupus erythematosus [8], and pemphigus [22]. Greater concentrations of this factor have also been detected in sera of individuals with many autoimmune diseases [10]. Additionally, in some of these diseases, administration of anti-MIF antibody has resulted in amelioration of the disease and inflammation [3, 16]. This is explained by the effects of MIF on inducing key proinflammatory cytokines such as interferons, IL-1, and TNF-α, and the enhancement in the survival and clonal expansion of autoreactive T cells [6, 11, 29]. Nevertheless, the pathogenesis of AA is different from other autoimmune diseases, since it involves an immune-privileged organ. Many immune-privileged sites such as the anterior chamber of the eye and the nails show a strong expression of MIF in a physiological state when there is no inflammation involved [13, 18, 37]. Similarly, it has been demonstrated that treatment with MIF can result in a decrease in the catalytic activities of NK cells [2, 28].

Tanking into account our findings with the studies from other immune-privileged organs, we believe that the role of MIF in the pathogenesis of AA may be more about preserving the immune privilege of anagen follicles than a proinflammatory role. We think that at some point of the disease process elevated tissue and serum levels of MIF may function as a proinflammatory factor but this could just reflect a secondary phenomenon, perhaps secondary to the elevated levels of glucocorticoids. However, more comprehensive studies with a larger sample size that also measures the serum and tissue levels of MIF could provide more insight into the causal relationship between AA and MIF.

Acknowledgements We wish to present our special thanks to Dr Azin Ayatollahi for her contributions that greatly assisted the research.

Author contributions FR, MMA, MA, and NJ performed the research. RMR, MMA designed the research study. FR, MA, and NJ analyzed the data. FR, MMA and RMR wrote the paper. All authors have read and approved the final manuscript.

Funding This research did not receive any specific Grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval (research involving human participants and/or animals) The study was approved by the Ethics commitee of Shahid Beheshti University of Medical Sciences.

Informed consent A written informed consent was obtained from all participants according to the declaration of Helsinki.

References

1. Apte RS, Sinha D, Mayhew E, Wistow G, Niederkorn J (1998) Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. J Immunol 160:5693–5696
2. Arcuri F, Cintorino M, Carducci A, Papa S, Riparbelli M, Mangioni S, Di Blasio A, Tosi P, Vigano P (2006) Human decidual natural killer cells as a source and target of macrophage migration inhibitory factor. Reproduction 131:175–182
3. Denkinger CM, Metz C, Fingerle-Rowson G, Denkinger MD, Forsthuber T (2004) Macrophage migration inhibitory factor and its role in autoimmune diseases. Arch Immunol Ther Exp (Warsz) 52:389–400
4. Donn R, Alourfi Z, De Benedetti F, Meazza C, Zeggini E, Lunt M, Stevens A, Shelley E, Lamb R, Ollier WE (2002) Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheumatol 46:2402–2409
5. Donn R, Shelley E, Ollier W, Thomson W, Group BPRS (2001) A novel 5′-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 44:1782–1785
6. Donn RP, Plant D, Jury F, Richards HL, Worthington J, Ray DW, Griffiths CE (2004) Macrophage migration inhibitory factor gene polymorphism is associated with psoriasis. J Invest Dermatol 123:484–487
7. Donnelly SC, Bucala R (1997) Macrophage migration inhibitory factor: a regulator of glucocorticoid activity with a critical role in inflammatory disease. Mol Med Today 3:502–507
8. Foote A, Briganti EM, Kipen Y, Santos L, Leech M, Morand EF (2004) Macrophage migration inhibitory factor in systemic lupus erythematosus. J Rheumatol 31:268–273
9. Gilhar A (2010) Collapse of immune privilege in alopecia areata: coincidental or substantial? J Invest Dermatol 130:2535–2537
10. Greven D, Leng L, Bucala R (2010) Autoimmune diseases: MIF as a therapeutic target. Expert Opin Ther Tar 14:253–264
11. Illescas O, Gomez-Verjan JC, García-Velázquez L, Govezensky T, Rodriguez-Sosa M (2018) Macrophage migration inhibitory factor – 173 G/C polymorphism: a global meta-analysis across the disease spectrum. Front Genet 9:55
12. Ito T, Ito N, Bettermann A, Tokura Y, Takigawa M, Paus R (2004) Collapse and restoration of MHC class-I-dependent immune privilege: exploiting the human hair follicle as a model. Am J Pathol 164:623–634
13. Ito T, Ito N, Saathoff M, Stampachiacchiere B, Bettermann A, Bulfone-Paus S, Takigawa M, Nickoloff BJ, Paus R (2005) Immunology of the human nail apparatus: the nail matrix is a site of relative immune privilege. J Invest Dermatol 125:1139–1148
14. Ito T, Ito N, Saatoff M, Hashizume H, Fukamizu H, Nickoloff BJ, Takigawa M, Paus R (2008) Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. J Invest Dermatol 128:1196–1206
15. Kang H, Wu W-Y, Lo BK, Yu M, Leung G, Shapiro J, McElwee KJ (2010) Hair follicles from alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. J Invest Dermatol 130:2677–2680
16. Leech M, Metz C, Bucala R, MorAnd EF (2000) Regulation of macrophage migration inhibitory factor by endogenous glucocorticoids in rat adjuvant-induced arthritis. Arthritis Rheumatol 43:827–833
17. Matia-García I, Salgado-Goytia L, Muñoz-Valle JF, García-Arellano S, Hernández-Bello J, Salgado-Bernabé AB, Parra-Rojas I (2015) Macrophage migration inhibitory factor promoter polymorphisms (-794 CATT 5–8 and-173 G> C): relationship with mRNA expression and soluble MIF levels in young obese subjects. Dis Markers 2015:461208
18. Meyer K, Klatte J, Dinh H, Harries M, Reithmayer K, Meyer W, Sinclair R, Paus R (2008) Evidence that the bulge region is a site of relative immune privilege in human hair follicles. Br J Dermatol 159:1077–1085
19. Mirzoyev SA, Schrum AG, Davis MD, Torgerson RR (2014) Lifetime incidence risk of alopecia areata estimated at 2.1 percent by rochester epidemiology project, 1990–2009. J Invest Dermatol 134:1141
20. Morales-Zambrano R, Bautista-Herrera LA, De la Cruz-Mosso U, Villanueva-Quintero GD, Padilla-Gutiérrez JR, Valle Y, Parra-Rojas I, Rangel-Villalobos H, Gutiérrez-Ureña SR, Muñoz-Valle JF (2014) Macrophage migration inhibitory factor (MIF) promoter polymorphisms (− 794 CATT5–8 and – 173 G > C): association with MIF and TNFα in psoriatic arthritis. Int J Clin Exp Med 7:2605
21. Murakami H, Akbar SMF, Matsui H, Onji M (2001) Macrophage migration inhibitory factor in the sera and at the colonic mucosa in patients with ulcerative colitis: clinical implications and pathogenic significance. Eur J Clin Invest 31:337–343
22. Namazi MR, Fallahzadeh MK, Shaghelani H, Kamali-Sarvestani E (2010) Marked elevation of serum macrophage migration inhibitory factor levels in patients with pemphigus vulgaris. Int J Dermatol 49:146–148
23. Ohwatari R, Inuyama Y, Fukuda S, Onoé K, Iwabuchi K, Nishihira J (2001) Serum level of macrophage migration inhibitory factor as a useful parameter of clinical course in patients with Wegener's granulomatosis and relapsing polychondritis. Ann Oto Rhinol Laryn 110:1035–1040
24. Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, Duvic M, King LE, McMichael AJ, Randall VA (2004) Alopecia areata investigational assessment guidelines-part II. J Am Acad Dermatol 51:440–447
25. Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, Kim H, Singh P, Lee A, Chen WV (2010) Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. Nature 466:113
26. Rajabi F, Drake L, Senna M, Rezaei N (2018) Alopecia areata: a review of disease pathogenesis. Br J Dermatol 179:1033–1048
27. Rajabi F, Amoli MM, Robati RM, Almasi-Nasrabadi M, Jabalameli N, Moravvej H (2019) The Association between Genetic Variation in Wnt Transcription Factor TCF7L2 (TCF4) and Alopecia Areata. Immunol Invest. https://doi.org/10.1080/08820139.2019.1597109
28. Repp AC, Mayhew ES, Apte S, Niederkorn JY (2000) Human uveal melanoma cells produce macrophage migration-inhibitory factor to prevent lysis by NK cells. J Immunol 165:710–715
29. Saeedi M, Amoli M, Robati R, Sayahpour F, Namazi N, Toossi P (2013) Macrophage migration inhibitory factor gene polymorphism is not associated with pemphigus vulgaris in Iranian patients. J Eur Acad Dermatol Venereol 27:1127–1131

30. Salem SA, Asaad MK, Elsayed SB, Sehsah HM (2016) Evaluation of macrophage migration inhibitory factor (MIF) levels in serum and lesional skin of patients with alopecia areata. Int J Dermatol 55:1357–1361
31. Shimizu T, Abe R, Ohkawara A, Nishihira J (1999) Increased production of macrophage migration inhibitory factor by PBMCs of atopic dermatitis. J Allergy Clin Immun 104:659–664
32. Shimizu T, Hizawa N, Honda A, Zhao Y, Abe R, Watanabe H, Nishihira J, Nishimura M, Shimizu H (2005) Promoter region polymorphism of macrophage migration inhibitory factor is strong risk factor for young onset of extensive alopecia areata. Genes Immun 6:285–289
33. Shimizu T, Mizue Y, Abe R, Watanabe H, Shimizu H (2002) Increased macrophage migration inhibitory factor (MIF) in the sera of patients with extensive alopecia areata. J Invest Dermatol 118:555
34. Shimizu T, Nishihira J, Mizue Y, Nakamura H, Abe R, Watanabe H, Ohkawara A, Shimizu H (2001) High macrophage migration inhibitory factor (MIF) serum levels associated with extended psoriasis. J Invest Dermatol 116:989–990
35. Shimizu T, Nishihira J, Mizue Y, Nakamura H, Abe R, Watanabe H, Ohkawara A, Shimizu H (2001) High macrophage migration inhibitory factor (MIF) serum levels associated with extended psoriasis. J Invest Dermatol 116:989–990
36. Stosic-Grujicic S, Stojanovic I, Maksimovic-Ivanic D, Momcilovic M, Popadic D, Harhaji L, Miljkovic D, Metz C, Mangano K, Papaccio G (2008) Macrophage migration inhibitory factor (MIF) is necessary for progression of autoimmune diabetes mellitus. J Cell Physiol 215:665–675
37. Streilein JW (2003) Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nat Rev Immunol 3:879
38. Younan DNA, Agamia N, Elshafei A, Ebeid N (2015) Serum level of macrophage migration inhibitory factor (MIF) in Egyptians with alopecia areata and its relation to the clinical severity of the disease. J Clin Lab Anal 29:74–79