

Research Article

Macrophage Migration Inhibitory Factor and Gene Polymorphism (rs755622G>C) in Unstable Vitiligo Patients

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Vitiligo pathogenesis is related to the macrophage migration inhibitory factor (MIF) protein. This study aimed to assess the lesional MIF levels and gene polymorphisms (rs755622G>C) in patients with vitiligo. To assess the consequences of combining narrow-band ultraviolet B with oral minipulse prednisolone as opposed to a combination with oral methotrexate on MIF levels in vitiligo patients, 50 unstable vitiligo patients and 50 controls were randomly chosen for comparison. MIF levels in skin homogenates and MIF (rs755622G>C) single nucleotide polymorphisms were assessed using the ELISA and the polymerase chain reaction restriction fragment length polymorphism (RFLP-PCR) techniques. We found significantly higher lesional MIF levels, a higher frequency of both (GC) and (CC) genotypes, and a significantly more frequent mutant allele (C) in patients than in controls. In addition, there was a significantly lower frequency of the allele (C) among patients who exhibited moderate to marked therapeutic improvement than among those who showed minimal to mild improvement. In conclusion, tissue MIF and gene polymorphisms were associated with vitiligo. In addition, oral corticosteroids, narrow-band ultraviolet B, methotrexate-targeted tissue MIF, and gene polymorphisms can improve unstable vitiligo.

1. Introduction

Vitiligo is the most common depigmenting skin disease, and patients with vitiligo often suffer from depression, anxiety, and many other psychological problems [1]. Many etiological factors have been reported in vitiligo, and their contributions to skin melanocyte destruction have also been reported. This includes the role of cellular and humoral immunity [2, 3]. Macrophages are a significant source of migration inhibitory factor (MIF), possibly contributing to vitiligo's etiopathogenesis. The MIF protein is (formed of 115 amino acids) an immune cell enhancer, prevents macrophage migration *in vivo*, and liberates many different cytokines, which play a crucial role in vitiligo development [4]. MIF promotes proinflammatory activity and increases the release of tumor

necrosis factor (TNF) by affecting the immune response via autocrine, paracrine, and endocrine routes. Also, MIF acts as a regulator against the immunosuppressive effects of glucocorticoids; therefore, MIF is considered to be a gene possibly associated with autoimmune-inflammatory diseases [5, 6]. Many treatment modalities have been implicated in the improvement of vitiligo, including ultraviolet B, systemic steroids, and methotrexate [7]. Few works in the literature show the possible role of MIF in vitiligo development. Consequently, we aim in this study to identify the presence of MIF levels in lesional tissues and the genetic polymorphisms associated with them (rs755622G>C) in vitiligo patients. In addition, to evaluate the effect of oral minipulse prednisolone combined with narrow-band ultraviolet B versus oral methotrexate combined with narrow-band ultraviolet B on

the patients' recovery results from one side and the MIF tissue levels and its gene polymorphism from the other side. So in this study, our primary objective was to measure the MIF concentrations in lesional tissues pre- and posttherapeutic options (oral minipulse prednisolone combined with narrow-band ultraviolet B versus oral methotrexate combined with narrow-band ultraviolet B) to determine its possible prognostic utility in vitiligo patients and also, to compare the outcomes of these two therapeutic options regarding the patients' response. The secondary objective was to investigate the role of MIF gene polymorphism (rs755622G>C) in development and response to therapy among patients with vitiligo. Such data can contribute in the future to the development of new treatment options and the diagnosis in vitiligo.

2. Patients and Methods

Fifty vitiligo patients with different degrees of severity and 50 controls were randomized through age and sex matching. Patients and controls were enrolled in accordance with the Declaration of Helsinki. The research was performed in the departments of dermatology and medical biochemistry at Qena University Hospital from May 2018 to May 2020 after receiving an acceptance and approval number (98, 2018). All participants signed and agreed to provide written consent at all stages of the trial and publish the data. Individuals with stable vitiligo, liver, and renal dysfunction; those under or previously under phototherapy; those on immunosuppressive drugs; and pregnant and lactating females were excluded from the present study. Group A patients received NB-UVB for three sessions weekly on nonconsecutive days for six months, plus a minipulse corticosteroid (prednisolone) of 0.1 mg/kg/day, taken on two consecutive days weekly. Group B patients received NB-UVB three sessions weekly on nonconsecutive days for six months, plus methotrexate (10 mg per week).

2.1. Randomization and Masking. Patients were randomly allocated (1:1) to receive either methotrexate plus conventional NB-UVB or a minipulse corticosteroid plus NB-UVB. Randomization was performed using an electronic case report system with blocks of different sizes (4, 6, and 8). Web-based automated randomization was used in the present study. While investigators and healthcare professionals were not blinded by the study drug assignment, patients and data interpreters were.

2.2. Dermatological Assessment of the Included Patients. All the participants were assessed for unstable vitiligo. Disease severity was assessed before and after treatment using the Vitiligo Area Severity Index (VASI), a standardized camera, and a graded scoring scale.

2.3. Blood and Tissue Samples. Blood samples (3 ml) were collected from all the patients and evacuated into EDTA tubes. All samples were frozen at -80°C until genotype

identification. Tissue samples were obtained from the vitiliginous areas and the skin of the volunteers using the dermatological punch technique with a diameter of 3 mm. All punched biopsies were homogenized using a Potter-Elvehjem rotor-stator homogenizer (glass or Teflon homogenizer) and preserved at -80°C for later evaluation of MIF concentrations in the tissues.

2.4. Measurements of Tissue MIF Levels. ELISA kits for MIF (catalog number: BYEK3015; Chongqing Biosciences Co., Ltd., China) were used for biochemical assays. After centrifugation and homogenization of skin samples at $822\times\text{g}$ for 20 minutes, the total protein content of each tissue sample was determined using commercial kits supplied by Spectrum Diagnostics, Egypt (Cat No. 310001) and a spectrophotometer (Chem-7, Erba Diagnostics Mannheim GmbH, Germany). The MIF concentration in each gram of tissue protein was then calculated.

2.5. Genetic Assays for MIF-173G>C (rs755622) Single Nucleotide Polymorphism. Blood samples were obtained from the participants according to the manufacturer's instructions (iNtRON Biotechnology, Inc., Made, Korea) and stored at -80°C . Genotyping of the MIF-173G>C (rs755622) SNP was performed using RFLP-PCR with the following primer sequences (supplied by Beijing SBS Genetech, China).

Forward primer was 5'-ACTAAGAAAGACCCGAGG C-3'. The reverse primer used was 5'-GGGGCACGTTGG TGTTCAC-3', which agrees with previously published studies [8, 9]. The PCR conditions used for amplification of the target SNP were as follows: 35 cycles of 10 minutes of initial denaturation at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds, and a final delay of 10 minutes. PCR products (Figure 1(a)) were 366 bp in size, using a 50 bp DNA ladder, digested at 37°C for three hours using Fast Digest AluI FD0014, Lot: 00147479; Thermo Fisher Scientific, and then loaded for gel electrophoresis on a 2% agarose gel stained with 5 μL ethidium bromide. The MIF restriction fragment lengths were two fragments for the wild-type genotype variant (GG) of 98 and 268 bp; three fragments of 205, 98, and 63 bp for the homozygous mutant genotype (CC); and four fragments of 268, 205, 98, and 63 bp for the heterozygous mutant genotype (GC) (Figure 1(b)). DNA samples from approximately 10%–15% of the participants were randomly selected to be re-genotyped to confirm the reproducibility of the genotyping results.

2.6. Statistical Analysis. The data were analyzed using SPSS version 26. A normality test (Kolmogorov–Smirnov and Shapiro–Wilk tests) was performed, and the data were not normally distributed. Categorical data were analyzed by number and percentage (N, %), whereas continuous data were described as the mean \pm standard deviation (mean \pm SD) or median and interquartile range (IQ). Categorical variables were compared using the chi-square test. The Mann–Whitney *U* test for nonparametric data demonstrated differences between the two groups. Differences

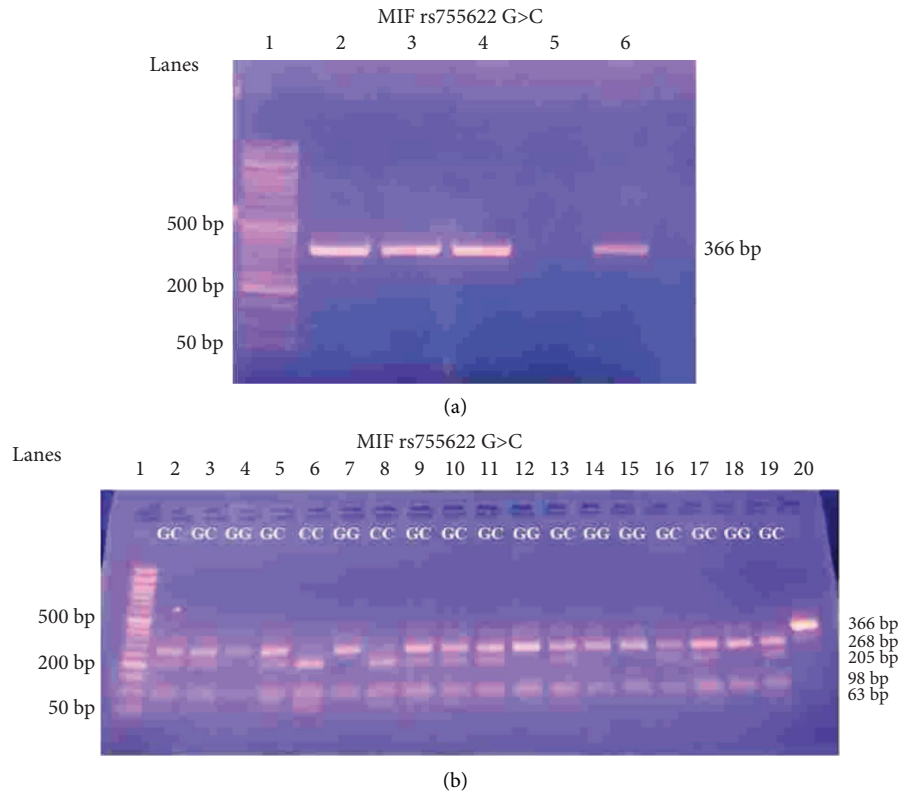


FIGURE 1: Gel electrophoresis of PCR and MIF products for the rs755622G>C SNP was performed using the PCR-RFLP method. Numbers refer to lanes. (a) Lane 1 shows a 50-bp DNA ladder; lanes (2–4, 6) represent amplified DNA segments (undigested PCR product) with a length of 366 bp. (b) Lane 1 shows a 50 bp DNA ladder; lanes 2, 3, 5, 9, 10, 11, 13, 16, 17, and 19 are heterozygous mutant (GC) genotypes with 268, 205, 98, and 63 bp bands; lanes 6 and 8 are homozygous mutant (CC) genotypes with 205, 98, and 63 bp bands; lanes 4, 7, 12, 14, 15, and 18 are wild-type genotypes (GG) with 268, 98 bp bands. Lane 20 represents an undigested PCR product with a 366-bp band.

between more than two groups were detected using the Kruskal–Wallis test for nonparametric data. Pre- and post-MIF results were compared for each group in the related samples using the Wilcoxon signed-rank test. Statistical significance was set at $p < 0.05$. The studied SNP followed the Hardy–Weinberg (HW) equation [10, 11].

2.7. Sample Size Calculation Was Carried Out Using G*Power 3 Software (Faul et al.) [12]. A calculated minimum sample of 88 respondents was needed (raised by 20% to compensate for dropouts and nonrespondents). The sample was randomly assigned to one of two equal groups (study group ($n = 44$)), unstable vitiligo patients, and (control group ($n = 44$)) age- and sex-matched patients. This was needed to detect an effect size of 0.5 [13] in the mean of the MIF level with an error probability of 0.05 and 80% power on a two-tailed test.

3. Results

3.1. Clinical Characteristics of the Studied Groups. There were no significant differences between the study groups regarding age, sex, Fitzpatrick skin scale score, height, body mass index (BMI), duration of illness, or distribution sites ($p > 0.05$), respectively. There was a positive family history of

vitiligo in both patient groups (20%). There were significant improvements in the VASI score of each patient group on follow-up visits ($p < 0.001$ for all), although there was an insignificant improvement in the VASI score between the two groups ($p > 0.05$). There was no significant difference in the regimentation patterns or improvement responses between the two patient subgroups ($p > 0.05$) (Figure 2 and 3).

3.2. Tissue MIF Levels among the Study Groups. Table 1 shows significantly higher lesional tissue levels of MIF among patients with vitiligo compared to the controls ($p = 0.002$), with the lowering of its tissue expression posttherapy, reaching an insignificant difference compared with the control level ($p > 0.05$). However, there was no significant difference in the lesional tissue levels of MIF between vitiligo patients who exhibited minimal to mild improvement compared to those with moderate to marked improvement ($p > 0.05$), although there was an important reduction in the tissue MIF levels when comparing pretherapy with the posttherapy effect in each of the two categories ($p > 0.05$).

3.3. Genotype and Allele Frequencies of MIF-173G>C (rs755622) SNP in the Study Groups and Their Relation to the Levels of MIF in Tissues. The frequency of the wild-type genotype (GG) was considerably lower among patients with

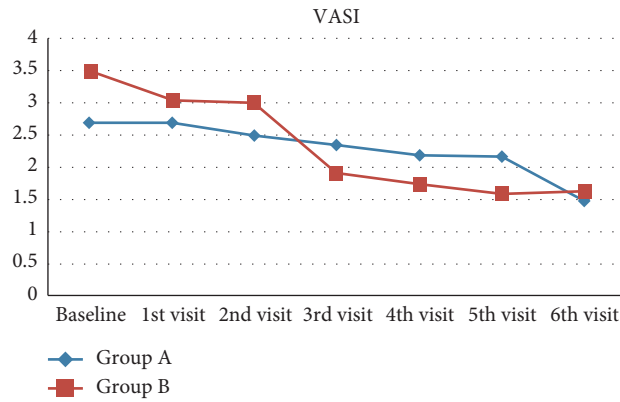


FIGURE 2: Comparison between the two study groups regarding VASI scores at all visits.

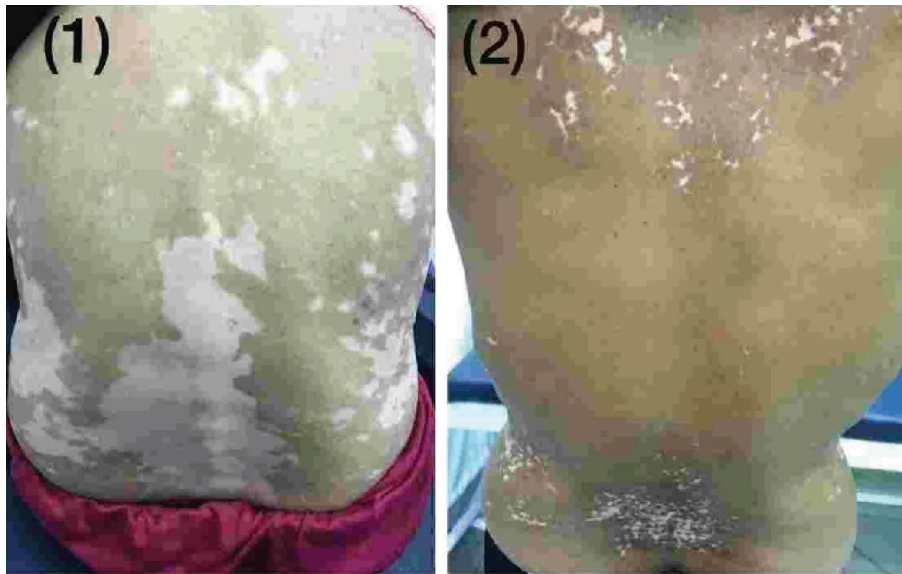


FIGURE 3: Back of a female vitiligo patient from group (A). (1) before treatment and (2) six months after treatment with NB-UVB plus minipulse prednisolone.

TABLE 1: Tissue levels of MIF among the study groups and tissue levels of MIF among the included patients in terms of response to therapy.

Variables	Cases (n = 50)	Controls (n = 50)	p-value
Pretherapy tissue MIF (median and IQR, $\mu\text{g/gm}$ tissue proteins)	0.36 (0.17–0.75)	0.18 (0.08–0.38)	0.002*
Posttherapy tissue MIF (median and IQR, $\mu\text{g/g}$ tissue proteins)	0.13 (0.04–0.56)	0.18 (0.08–0.38)	0.235
p value	<0.001*	—	—

Variables	Cases (n = 50)		p-value
	Minimal to mild improvement (n = 25)	Moderate to marked improvement (n = 25)	
Pretherapy tissue MIF (median and IQR, $\mu\text{g/g}$ tissue proteins)	0.29 (0.12–0.50)	0.24 (0.13–0.60)	0.617
Posttherapy tissue MIF (median and IQR, $\mu\text{g/g}$ tissue proteins)	0.16 (0.06–0.37)	0.18 (0.058–0.59)	0.424
p value	<0.001*	<0.001*	—

The Mann–Whitney *U* test was used for continuous data. **P* value <0.05 is considered significant.

TABLE 2: Genotypes and allele frequencies of MIF rs755622G>C nucleotide polymorphism among the study groups.

Study groups	Variables																	
	MIF rs755622G>C genotypes										MIF rs755622G>C alleles							
	GG		GC		CC		GG+GC		CC		GG		GC+CC		G		C	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Cases (<i>n</i> = 50)	19	38	25	50	6	12	44	88	6	12	19	38	31	62	63	63	37	37
Controls (<i>n</i> = 50)	37	74	9	18	4	8	46	92	4	8	37	74	13	26	83	83	17	17
<i>p</i> value (χ^2)	0.001** (13.715)				0.505 (0.444)				<0.001** (13.149)				0.001** (10.147)					
OR (95% CI)	—				0.638 (0.168–2.413)				0.215 (0.092–0.505)				0.349 (0.180–0.675)					

The Chi-squared test was used for categorical variables. ***P* value <0.01 is considered significant.

vitiligo (38%), than among healthy volunteers (74%), and there was a considerably higher frequency of both heterozygous (GC) and homozygous mutant (CC) genotypes among patients with vitiligo (50% and 12%, respectively) than among controls (18% and 8%, respectively; $p = 0.001$). Using the recessive model, there was an increasing frequency of (GC + CC) and a markedly lower frequency of (GG) among patients with vitiligo (62% and 38%, respectively) compared to controls (26% and 74%, respectively; $p < 0.001$). There was a considerably higher mutant allele (C) frequency among patients (37% compared with the control 17%) and a significantly higher normal allele (G) frequency among controls (83% compared with 63%) ($p = 0.001$), indicating that the mutant allele (C) was a risk factor for vitiligo development (Table 2). Within the patient group, there was a significantly higher wild genotype (GG) and significantly lower heterozygous and homozygous mutant genotypes (GC and CC, respectively) among vitiligo patients who exhibited moderate to marked improvement after therapy (68%, 28%, and 4%, respectively) compared to those who showed minimal to mild improvement (44%, 40%, and 16%, respectively; $p = 0.027$). In addition, there was a considerably higher frequency of a normal allele (G) and a considerably lower frequency of the mutant allele (C) among vitiligo patients who exhibited moderate to marked improvement after therapy (82% and 18%, respectively) compared to those who showed minimal to mild improvement (64% and 36%, respectively) ($p = 0.027$), with an OR (95% CI) of 0.349 (0.180–0.675), indicating that a mutant allele (C) is a risk factor for vitiligo development (Table 3). There were insignificant tissue MIF levels in the included patients in terms of various MIF genotypes ($p > 0.05$).

4. Discussion

Few studies have investigated the role of MIF in unstable vitiligo. Narrow-band ultraviolet B, oral minipulse prednisolone, and oral methotrexate have been used to lower MIF expression in unstable vitiligo. Therefore, clinicians should recognize MIF as a risk factor for vitiligo and target it in unstable vitiligo. In this study, we aimed at assessing MIF levels in lesional tissues and its gene polymorphisms (rs755622G>C) in vitiligo patients and their relationship with treatment with oral minipulse prednisolone combined with narrow-band ultraviolet B versus oral methotrexate

combined with narrow-band ultraviolet B. In our study, we found that patients with vitiligo who received NB-UVB plus minipulse prednisolone for 6 months showed significant VASI score improvement from the baseline visit to the 6th visit. This is due to the combined actions of NB-UVB and minipulse prednisolone, as NB-UVB stimulates melanocytes to cause repigmentation and minipulse prednisolone acts on MIF. Moreover, our results showed that patients with vitiligo who received NB-UVB plus low-dose methotrexate for 6 months demonstrated significant statistical differences in VASI score improvement from the baseline visit to the 6th visit. This is due to the combined actions of NB-UVB and methotrexate, as NB-UVB stimulates melanocytes to cause repigmentation, and methotrexate may suppress MIF and tumor necrosis factor- α . There were no significant differences between the two study groups in terms of the VASI score or improvement. In agreement with our results concerning this improvement in the VASI score, an oral minipulse of betamethasone combined with NB-UVB appears effective in inducing repigmentation [14–19]. A decrease in the depigmented area of the vitiligo lesion after 6 months of taking oral minipulse betamethasone and NB-UVB was documented [20]. These agree with the results of the present study regarding the significant improvement. Our results are comparable with those of another study using oral minipulse betamethasone along with NB-UVB, which found marked, moderate, and mild improvements in patients with vitiligo [21]. In our results, there were considerably higher lesional tissue levels of MIF among vitiligo patients compared to controls. This indicated that MIF may play a role in the development of vitiligo, with the lowering of its tissue expression posttherapy reaching an insignificant difference compared to the control level. This was due to the immunosuppressive actions of NB-UVB, minipulse prednisolone, and methotrexate. Serarslan et al. showed that there was no relationship between the VASI and MIF levels in the serum of patients with vitiligo [22]. In agreement with our results, Ma L et al. showed significantly higher levels of MIF protein in histopathological samples and MIF mRNA levels in patients with vitiligo than in healthy tissue [23]. In their work, Farag et al. showed that the levels of MIF mRNA and serum MIF concentrations were increased in vitiligo patients in comparison to controls [24]. Many cytokines, including the MIFrs755622

TABLE 3: Genotypes and allele frequencies of MIF rs755622G>C single nucleotide polymorphism among the included cases in terms of response to therapy.

Study groups	Variables																	
	GG				GC				MIF rs755622G>C genotypes				MIF rs755622G>C alleles					
	No.	%	No.	%	No.	%	No.	%	CC	CG	GC	CC	GG	GC+CC	G	C		
Minimal to mild improvement (<i>n</i> = 25)	11	44	10	40	4	16	21	84	4	16	11	44	14	56	32	64	18	36
Moderate to marked improvement (<i>n</i> = 25)	17	68	7	28	1	4	24	96	1	4	17	68	8	32	41	82	9	18
<i>p</i> -value (χ^2)	0.027* (7.230)				0.046* (4.000)				0.016* (5.844)				0.004** (8.219)					
OR (95% CI)	—				0.219 (0.044–1.088)				0.37 (0.164–0.836)				0.390 (0.203–0.750)					

The Chi-squared test was used for categorical variables. **P* value <0.05 is considered significant. ***P* value <0.01 is considered significant.

(-173G/C) polymorphism, have been reported to be documented in the development of some diseases such as rheumatoid arthritis, psoriasis vulgaris, and systemic lupus erythematosus [25, 26]. In documented research, the levels of the MIF-173C allele were also associated with an increase in serum MIF [27]. In our results, there was a considerably higher mutant allele (C) frequency among patients in comparison with the control, with a significantly higher normal allele (G) frequency among controls compared with the included patients, indicating that mutant allele (C) can be a risk factor for vitiligo development. In addition, there was a considerably higher frequency of a normal allele (G) and a considerably lower frequency of a mutant allele (C) among vitiligo patients who exhibited moderate to marked improvement following therapy compared to those who showed minimal to mild improvement, indicating that the mutant allele (C) can be considered a genetic factor affecting the individual response to vitiligo therapy. Similar to other studies, the current study showed that the MIF-173G/C frequency in unstable vitiligo patients was higher than that in stable nonsegmental vitiligo patients [28]. A relative limitation is the small sample size of this study; therefore, larger-scale studies to support the conclusions of the present study are advised. In our conclusion, we found that the tissue MIF and its gene polymorphism (rs755622G>C) among patients with vitiligo may have a role in disease progression and therapies aiming to treat vitiligo and are significantly associated with the response of these patients to therapy.

5. Conclusion

Tissue MIF and gene polymorphisms are associated with vitiligo. Furthermore, oral corticosteroids, narrow-band ultraviolet B, methotrexate-targeted tissue MIF, and gene polymorphisms can improve unstable vitiligo. As a result, clinicians may recognize MIF as a risk factor for vitiligo and target it to improve unstable vitiligo.

5.1. Study Limitations. The relatively small size was the main study limitation so findings of the current work need to be confirmed using larger-scale future research studies.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Hassan M. Ibrahim and Soheir Abdel-Hamid wrote, designed, and reviewed the manuscript. Eisa M. Hegazy contributed to the data interpretation. Moustafa A. El-Taieb conducted the data analysis. Essam A. Nada, Mohammed H. Hassan, Ali Younis, and Mahmmoud Ali performed the

practical parts of the research. All authors have approved the submitted manuscript.

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