Soluble CD27 and MIF as possible serum biomarkers of vitiligo activity in Egyptian patients in Sharkia Governorate

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Abstract

Contrary to other inflammatory skin disorders like psoriasis or atopic dermatitis, vitiligo does not present with distinct inflammatory symptoms that can be easily evaluated by clinical examination. Identification of a putative biomarker to inform early and accurate treatment responses could be of considerable value. This study aims to validate levels of serum soluble CD27 (sCD27) and macrophage Migration Inhibitory Factor (MIF) as biomarkers of vitiligo to improve the quality of disease management. This cross-sectional study was conducted on 32 vitiligo patients, stratified into two subgroups of 22 active and 10 stable vitiligo patients; the stable group containing 1 segmental and 9 non-segmental presentations, and 32 matched healthy individuals as the control group. Of the 32 patients in the study, 21 were female and 11 were male with a median age of 30 years. The measurements of the study parameters of sCD27 and MIF in the serum were carried out through blood sampling and followed up for three months at one-month intervals for stable vitiligo cases. Mean serum levels of sCD27 and MIF were significantly higher in vitiligo patients than in the control group. A positive correlation was observed in active vitiligo cases between both serum MIF and sCD27 levels and the spreading item of Vitiligo European Task Force (VETF) score as an indicator of disease activity. Serum sCD27 and MIF levels in vitiligo patients were observed to be higher than that of controls with greater correlation found for sCD27 with disease activity.

Introduction

Vitiligo is a depigmentation disorder of the skin and underlying mucous membranes with probable genetic and immune/autoimmune origins and results in typically localized depigmented macules and skin sections due to progressive melanocyte loss.[1]

Due to the lack of a distinct phenotype like erythema or scaling, early diagnosis of vitiligo has proven to be difficult. The search for a predictive biomarker could confirm the active disease and predict future disease progression.[2]

Clinical management of vitiligo is difficult due to the unpredictable nature of disease progression, encompassing periods of stability, and activation. Biomarker analysis could pave the way for better patient follow-up and predict the course of future disease progression[3]

The clinical criteria defining stable vitiligo include non-progressive old lesions within two years preceding examination, no new lesions developed in the same period, absence of Koebner phenomenon, re-pigmentation of depigmented areas either spontaneously or upon medical intervention, and positive Minigraft test with lack of 'Koebnerization' at donor site.[4]

Materials and Methods

The present study was performed at Zagazig University hospitals with approval from the Institutional Review Board (IRB) vide approval number 3584 on May 4, 2017. Thirty-two vitiligo patients (22 with active disease, 10 with stable disease) were enrolled in this cross-sectional study. Stable cases comprised of 1 segmental and 9 non-segmental vitiligo patients and females (21) were in majority (11 males). The inclusion criteria required patients to be treatment-naïve for the preceding six weeks before blood analysis. Patients with other dermatologic, autoimmune or systemic comorbidities were excluded from the study and informed consent was obtained from all participants.

Assessment of Disease Activity

The Vitiligo European Task Force (VETF) scoring system was used for assessment of disease activity in the study participants, adapted from Taieb and Picardo.[5]

A double-antibody sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method using commercial kits (SunRed® Human MIF ELISA Kit and SunRed® Human Tumor Necrosis Factor Superfamily, Member 7 (TNFSF7/CD27) ELISA Kit; SRB, Shanghai, China) were used to assess MIF and sCD27 levels in serum separated from blood samples. Assessments were conducted by the experts blinded to subject status.

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Declaration: We state that Dr. Mohamed Ibrahim EL-Ghareeb as a corresponding author has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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ELISA was carried out following the manufacturer's instructions. The microplate wells were coated with antigen first, followed by the primary antibody, and then enzyme-conjugated secondary antibody. Addition of a substrate that reacts with the enzyme resulted in a colored product indicating a positive reaction.

Data collected through basic clinical examination, and laboratory investigations were coded, entered, and analyzed first using Microsoft Excel and then imported into Statistical Package for the Social Sciences (SPSS Version 20.0) software for further analysis.

Results

The mean serum levels of MIF in vitiligo patients were found to be statistically significantly higher than that of the control group (1.31 ±0.68 ng/mL vs. 0.93 ±0.37 ng/mL; P=0.01), and a similar trend was observed for serum sCD27 levels for vitiligo patients as compared to the control group (163.91 ±58.19 ng/mL *vs* 107.31 ±49.75 ng/mL; P<0.001).

Mean VETF score in active vitiligo cases was 18.75 compared to 17.3 for stable vitiligo presentations. However, no significant difference was observed in the serum levels of sCD27 and MIF between active

and stable vitiligo cases (Table 1).

For the active cases, a statistically significant positive correlation was observed between both serum MIF and sCD27 levels and the spreading item of VETF score as an indicator of disease activity (Table 2).

A statistically significant increase in the frequency of clinical regression at the third and fourth visits as compared to the second visit was observed in stable vitiligo cases. Lower serum sCD27 levels were seen with the clinical regression in stable cases but were not statistically significant, while serum MIF levels did not correlate at all with clinical regression (Table 3). The sensitivity of serum MIF in patients with vitiligo correlating with the disease state at a cut off of 0.85 ng/mL was 78.1%, with 75% specificity, 76.6% accuracy, 75.8% positive predictive value and 77.4% negative predictive value.

Serum sCD27 demonstrated an 81.2% sensitivity to vitiligo disease state at a cut off of 125 ng/mL and 78.1% specificity, 80.6% accuracy, 78.8% positive predictive value, and 80.6% negative predictive value. Similar parameters recorded with regards to the spreading item of the VETF score for MIF and sCD27 were as follows: cut off= 1.43 ng/mL and 172 ng/mL; sensitivity= 72.2% and 77.8%; specificity= 71.4% and 78.6%; and accuracy= 71.9% and 78.1%.

Discussion

Overall, a statistically significant increase in serum MIF and sCD27 levels was seen among patients with vitiligo as compared to the control group.

MIF was originally characterized as a chemotactic lymphokine that attracts macrophages at inflammatory loci. MIF regulates cell-mediated immunity by activating macrophages *in vivo*.[6]

Lesions in vitiligo are also densely populated by macrophages, with increased numbers found in pre-lesional skin. A probable explanation for this finding may be that macrophages help in clearing the cytotoxic T lymphocyte-mediated apoptotic melanocytes.[7]

These findings are concordant with a previous study by Serarslan *et al*.[8] indicating that MIF may have a role in the pathogenesis of vitiligo, which assessed the serum samples in 30 subjects with vitiligo and matched healthy controls. The higher mean serum MIF level (1.31±0.68 ng/mL) as compared to controls (0.93±0.37 ng/mL) supports this theory.

In another study, serum MIF concentrations in vitiligo patients were also shown to

Table 1. Relation between serum levels of MIF and sCD27 and stability among the patient group

Variable	Active (n=22)	Stable (n=10)	MW	P
MIF				
Mean ±SD	1.35 ±0.78	1.22 ±0.41	0.27	0.79
Median	115.5	115		NS
Range	0.75-4.4	0.66-2.1		
s CD27				
Mean ±SD	170.82 ±64.78	148.7 ±38.65	1.02	0.31
Median	159.5	148		NS
Range	66-399	83-216		

SD: standard deviation; MW: Mann-Whitney test; NS: non-significant (p>0.05).

Table 2. Correlation between MIF and CD27 and age, duration, and clinical data among active cases.

Variable	MIF (n=22)		CD 27 (n=22)	
	r	P	r	P
Extension (%) item of VETF	0.24	0.28 NS	0.14	0.51 NS
Stage item of VETF	0.27	0.22 NS	0.17	0.44 NS
Spread item of VETF	0.62	0.002**	0.74	<0.001**
Total VETF score	0.30	0.17 NS	0.18	0.45 NS

r: Pearson's and Spearman's correlation; NS: Non-significant (p>0.05); **: Highly significant (p>0.01).

Table 3. Relation between MIF and CD27 and state among the stable cases at different times of follow up.

Variable	Visit	State	N	Mean ±SD	Median	Range	Test	P
MIF	2nd	Stable	8	1.26±0.39	1.2	0.7–1.87	M	0.79
		Regression	2	1.18 ±0.46	1.18	0.85–1.5	W	NS
							0.26	
	3rd	Stable	3	1.18 ±0.17	1.25	1–1.3	F	0.70
		Regression	4	1.38 ±0.35	1.35	1–1.8	0.39	NS
		Progression	1	1.25 ±0	1.25	1.25		
	4th	Stable	3	1.45 ±0.39	1.65	1–1.7	K	0.28
		Regression	4	1.56 ±0.63	1.5	1–2.25	2.58	NS
		Progression	1	2.55±0	2.55	2.55		
sCD27	2nd	Stable	8	143.5 ±32.9	145	103–186	t	0.55
		Regression	2	128±16.97	128	116–140	0.63	NS
	3rd	Stable	3	105 ±46	105	59–151	K	0.26
		Regression	4	123 ±12.3	119.5	113–140	2.27	NS
		Progression	1	154 ±0	154	154		
	4th	Stable	3	126 ±21.79	116	111–151	F	0.13
		Regression	4	117 ±19.61	115.5	95–142	3.01	NS
		Progression	1	174 ±0	174	174		

SD: Stander Deviation; t: Independent t-test; MW: Mann-Whitney test; F: ANOVA; K: Kruskal-Wallis test; NS: Non-Significant (P>0.05).

be significantly elevated in their serum, Peripheral Blood Mononuclear Cells (PBMCs), and lesional skin.[9]

In the present study, significantly higher levels of mean serum sCD27 were found in patients with vitiligo as compared to the control group ($P < 0.001$).

A member of the tumor necrosis factor receptor superfamily, sCD27 is expressed on T, B, and natural killer cells and mediates a number of immunological processes like lymphocyte survival, increased T-cell proliferation, and memory cell formation by binding to its ligand CD70. sCD27 is released into the bloodstream from the surface of activated lymphocytes.[2] Although serum sCD27 level is regarded as a biomarker of immune activation and disease burden in various inflammatory disorders, it is unclear whether sCD27 plays a functional role in these conditions or is a by-product of T-cell activation.[10] The sCD27 involvement in the pathogenesis of vitiligo may be due to the fact that vitiligo is a T–cell-mediated autoimmune disease.[11] In a cross-sectional study on vitiligo patients, serum sCD27 level was significantly correlated with the disease state.[2] Cellular immunity has been implicated in playing a role in the pathogenesis of vitiligo, since both helper and cytotoxic T cells promote a Th1 response with the secretion of TNF-α and interferon IFN-γ.[12] Looking at the active cases in this study, serum MIF and sCD27 levels were positively significantly correlated with total VETF score, especially with the spread item, as well as with each other.

No statistical significance was observed for the correlation of serum sCD27 level with the clinical regression and progression in the stable cases, and serum MIF levels were unassociated with clinical regression in the stable cases. Thus, serum sCD27 may be a better indicator of disease activity given its correlation with disease progression and regression, than serum MIF levels. Further studies are required to validate this hypothesis.

It is possible that the elevation of serum sCD27 occurs at an earlier time point in the course of the disease than serum MIF. This is supported by the fact that sCD27 is a by-product of T cell activation, which is an earlier step in the pathway of cytotoxic T–cell-mediated destruction of melanocytes in vitiligo, while serum MIF may represent an end-stage of the pathogenesis, indicating the scavenging of apoptotic melanocytes already destroyed by cytotoxic T cells by macrophages.

Statistically significant increases in serum MIF and sCD27 were seen at a higher frequency in acrofacial as compared to other types of vitiligo. This finding is concordant with a previous study by Serarslan[8] and colleagues where a significant difference in serum MIF levels was seen in patients with acral-acrofacial vitiligo from those with generalized vitiligo.[8]

Conclusions

Active cases of vitiligo show increased levels of both sCD27 and MIF, indicating that they may be reliable serum biomarkers and sCD27 (and MIF to a lesser extent) may have a predictive capacity for diagnosing the progression of vitiligo lesions.

Limitations

The small sample size and unequal distribution of patients with active and stable disease states in this study is a major limitation. Additionally, the follow-up period, for stable vitiligo patients, needs to be longer than three months in order to detect reactivation and/or regression of the disease. This would also highlight the ability of these serum biomarkers as predictors of disease activity.

References

1. Kyriakis KP, Palamaras I, Tsele E, et al. Case detection rates of vitiligo by gender and age. Int J Dermatol 2009; 48:328–9.
2. Speeckaert R, Lambert J, Van Geel N. Clinical significance of serum soluble CD molecules to assess disease activity in vitiligo. JAMA Dermatol 2016;152: 1194–1200.
3. Speeckaert R, Speeckaert M, De Schepper S, van Geel N. Biomarkers of disease activity in vitiligo: a systematic review. Autoimmun Rev 2017;16:937–45.
4. Falabella R, Arrunategui A, Barona MI, Alzate A. The minigrafting test for vitiligo: detection of stable lesions for Melanocyte transplantation. J Am Acad Dermatol 1995;32:228–32.
5. Taieb A, Picardo M. Clinical practice. Vitiligo. N Engl J Med 2009;360:160–9.
6. Shimizu T. Role of macrophage migration inhibitory factor (MIF) in the skin. J Dermatol Sci 2005;37: 65–73.
7. Rezaei N, Gavalas NG, Weetman AP, Kemp EH. Autoimmunity as an aetiologic factor in vitiligo. J Eur Acad Dermatol Venereol 2007;21:856–76.
8. Serarslan G, Yönden Z, Söðüt S, et al. A Macrophage migration inhibitory factor in patients with vitiligo and relationship between duration and clinical type of disease. Clin Exp Dermatol 2010;35: 487–90.
9. Ma L, Xue H-B, Guan X-H, et al. Relationship of macrophage migration inhibitory factor levels in PBMCs, lesional skin and serum with disease severity and activity in vitiligo vulgaris. Braz J Med Biol Res 2013;46:460–4.
10. Ciccarelli BT, Yang G, Hatjiharissi E, et al. Soluble CD27 is a faithful marker of disease burden and is unaffected by the rituximab-induced IgM flare, as well as by plasmapheresis, in patients with Waldenström's macroglobulinemia. Clin Lymphoma Myeloma 2009;9:56–8.
11. Ezzedine K, Eleftheriadou V, Whitton M, van Geel N. Vitiligo. Lancet 2015;386:74–84.