ORIGINAL ARTICLE

Study of the Serum Level of the Monokine Induced by Interferon Gamma (MIG) in Vitiligo Patients

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ABSTRACT

Background: Monokine induced by interferon gamma (MIG), is a member of the CXC chemokine superfamily. Elevated serum MIG level has been found in some autoimmune diseases. The MIG costimulates B cells *in vitro* and *in vivo*. In addition, MIG has an important role in T-cell activation and survival. Therefore, this cytokine has been proposed to play a role in pathogenesis of vitiligo.

Objective: The aim of this work was to study the serum level of MIG in patients with vitiligo to evaluate its possible role in its pathogenesis.

Patients and Methods: This study included 40 patients with vitiligo and 30 healthy people who served as a control group. Serum samples were taken from all patients and controls for detection of serum MIG level by enzyme-linked immunosorbent assay (ELISA).

Results: The mean serum level of MIG was 231.40 pg/ml in vitiligo patients and 78.33 pg/ml in the control group. A significant increase was found in the mean serum MIG level in vitiligo patients when compared to the control group ($p = 0.001$). No significant difference was found in MIG level with different clinical types of vitiligo ($p = 0.065$). Serum MIG level was significantly correlated with the activity of vitiligo as determined by vitiligo disease activity score ($r=0.366$, $p = 0.001$).

Conclusions: MIG could be implicated as an important cytokine in the pathogenesis of vitiligo. MIG could be a useful marker for assessing vitiligo activity and may open the way for further therapeutic approaches for vitiligo. To the best of our knowledge, this is the first work to study serum MIG level in vitiligo.

INTRODUCTION

Monokine induced by gamma interferon (MIG) is a small cytokine belonging to the CXC chemokine family and shares binding to CXC receptor 3 (CXCR3). The MIG is expressed by monocytes, macrophages, antigen presenting cells (APC), eosinophils, endothelial cells, and B cells.[1] It plays a key role in leukocyte trafficking, acting on activated CD4+ Th1 cells, CD8+ T cells, interleukin (IL)-2 activated T lymphocytes, B lymphocytes, and natural killer (NK) cells.[2] In humans, the serum MIG level was found to be elevated in patients with systemic autoimmune diseases such as rheumatoid arthritis,[3] Graves disease, allergic asthma, systemic lupus erythematosus, ulcerative colitis, psoriasis, systemic sclerosis,[4] and alopecia areata.[5] Its level is often correlated to disease progression.[3]

Vitiligo is an acquired idiopathic pigmentary disorder characterized by circumscribed depig-

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mented macules resulting from loss of cutaneous melanocytes. The aetiology of the disease remains obscure,[6] although autoimmunity has been suggested to play a role in the development of the disease. It is frequently associated with other autoimmune disorders where both autoantibodies and autoreactive T lymphocytes that target melanocytes have been detected.[7]

The aim of this work was to study the serum level of the MIG in patients with vitiligo to evaluate its possible role in the pathogenesis of the disease.

PATIENTS AND METHODS

The present study was carried out on 40 patients with active vitiligo and 30 healthy subjects who served as a control group. The control group subjects were selected so that they had no positive family history of vitiligo. They were selected from the Outpatient Clinics of Dermatology and Venereology Department, Tanta University Hospitals, Tanta, Egypt. The participants were subjected to:

Full history taking: including onset, course, duration of the disease, previous treatments, history of drug intake such as corticosteroids, family history of the disease, and past history of other skin or systemic diseases.

Clinical examination: to determine the clinical distribution of vitiligo and to look for clinical manifestations suggestive of systemic diseases including autoimmune diseases.

The patients were classified according to the clinical picture and the disease activity was assessed using the vitiligo disease activity (VIDA) score (from +1 to +4).[8] Patients having systemic autoimmune diseases, other skin diseases, treatment for vitiligo within the last month before being involved in the study, and pregnant or lactating women were excluded from the study.

Measurement of the serum MIG level

3 ml of fresh venous blood was collected in plain tubes and then centrifuged. Serum was retrieved using sterile pipettes and stored at -20°C till the time of the assay. Hemolysed or lipemic samples were excluded. Human MIG-ELISA (Bender Med Systems Gmb H campus Vienna Biocenter 1030 Vienna, Austria) was used for quantitative detection of human MIG in cell culture supernatants, human serum, plasma, or other body fluids. The assay was performed according to the manufacturer's instructions.

Statistical Analysis

Statistical presentation and analysis of the present study was conducted by SPSS V17 (Statistical Program for Social Science, Version 17) using mean, standard deviation (SD), standard error (SE), Mann-whitney, Linear correlation coefficient, Chi-square, analysis of variance (ANOVA) tests and unpaired student T-test. p value (probability index) < 0.05 was considered significant, $p \leq 0.001$ was considered highly significant, and p value > 0.05 was considered non- significant.

RESULTS

Demographic and clinical results

The patients' group included 20/40 females (50%) and 20/40 males (50%). Their ages ranged from 5 to 65 years with a mean of 35±20.24 years. The mean disease duration was 4.12±2.81 months. The control group included 14/30 females (47%) and 16/30 males (53%). Their ages ranged from 15 to 50 years with a mean of 33.26±10.53 years. Vitiligo was generalized in 12/40 (30%) of patients, localized in 10/40 (25%) of patients, and acrofacial in 18/40 (45%) of patients (Table 1). VIDA score was +1 in 6/40 (15%) of patients, +2

in 6/40 (15%) patients, +3 in 12/40 (30%) of patients, and +4 in 16/40 (40%) of patients (Table 2).

Table 1 Clinical types of vitiligo in the studied patients (n = 40)

	Generalized	Localized	Acrofacial
Number of patients (%)	12 (30%)	10 (25%)	18 (45%)

Table 2 VIDA scores in the studied patients (n = 40)

	VIDA score			
	+1	+2	+3	+4
Number of patients (%)	6	6	12	16

Laboratory results

Serum MIG level ranged from 65 to 482 with a mean of 231.4±51.24 pg/ml in vitiligo patients and ranged from 54-136 pg/ml with a mean of 78.33±11.67 in the control group. The difference between both groups was statistically highly significant (p = 0.001) (Fig. 1).

The mean serum MIG Level was 160.7±25.44 pg/ml in the generalized type (range: 65-234 pg/ml), 210.3±36.95 pg/ml in the localized type (range: 93-482 pg/ml), and 145.3±68.33 pg/ml in the acrofacial type (range: 72-304 pg/ml). The difference between the mean serum MIG level between different clinical types of vitiligo was not statistically significant p = 0.065 (Fig. 2).

The mean serum MIG level was 75±15.6 pg/ml in patients with +1 VIDA score, 131.3±71.1pg/ml in patients with +2 VIDA score, 274.1±85.6 pg/ml in patients with +3 VIDA score and 295.5±48.6 pg/ml in patients with +4 VIDA score. The difference between them was statistically significant p = 0.001 (Fig. 3).

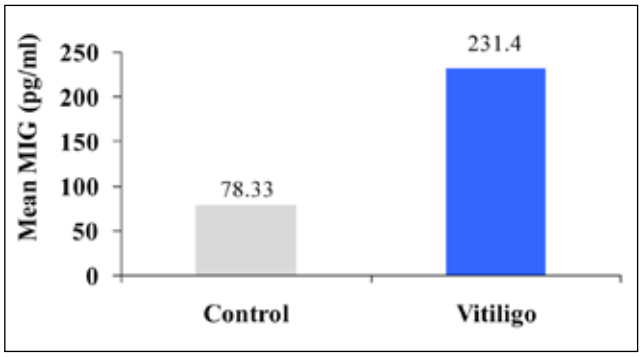

Fig. 1 Serum MIG level in vitiligo patients versus the control group. There is a highly significant increase of serum MIG level in vitiligo patients versus controls (p = 0.001).

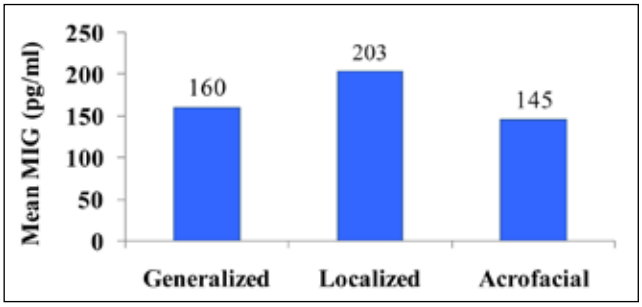

Fig. 2 Serum MIG level in different clinical types of vitiligo. The mean serum MIG level in different clinical types of vitiligo is statistically insignificant (p = 0.065).

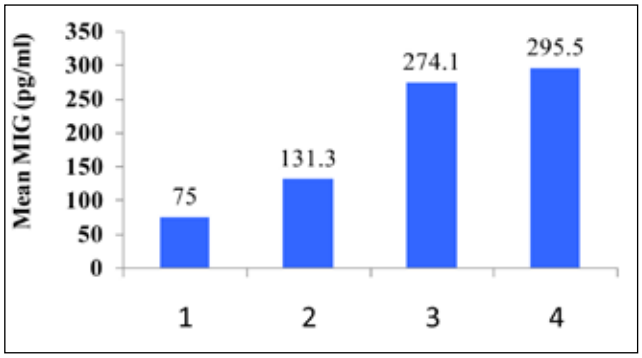

Fig. 3 Serum MIG levels versus positive VIDA scores. The difference between them was highly statistically significant (p = 0.001).

Clinico-serological correlation

There was a significant positive correlation between the serum MIG level and vitiligo activity, as indicated by VIDA scores (Table 3, Fig. 4). There was no significant correlation between the serum MIG level and either the age and gender of patients, the duration of disease, or the clinical type (Table 6).

Table 3 Correlation of serum MIG in vitiligo patients with age, gender, duration, VIDA score, and clinical type

	MIG in vitiligo patients	
	r	**p value**
Age	0.163	0.491
Gender	-0.223	0.346
Duration	-0.248	0.292
Clinical types	0.338	0.145
VIDA scores	0.366	0.001*

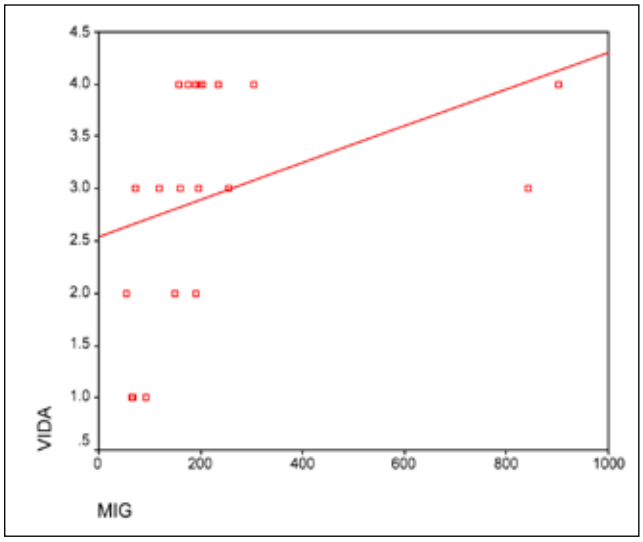

Fig. 4 Correlation of serum MIG levels with positive VIDA scores. Serum MIG level is significantly correlated with VIDA scores (r = 0.366, p = 0.001).

DISCUSSION

There is an evidence that both cellular and humoral immunity, are involved in disappearance of vitiligo-related melanocytes where both cytotoxic T cells and B cells were found to be simultaneously increased in patients with recent onset vitiligo.[9,10] A concurrent elevation in leucocyte inhibition factor released by activated T lymphocytes and circulating IgG antibodies to human melanocytes was seen in vitiligo patients. This raised the suggestion that a T cell mediated B cell-activation have occurred.[9]

IgG antibodies to human melanocytes are able to destroy melanocytes both in vitro and in vivo. Antibodies to human melanocytes have been found in sera from patients with vitiligo, but not in normal sera.[11] The level of these antibodies correlated to the extent of depigmentation as well as to the disease activity in vitiligo.[12] Their binding to cultured melanocytes increased along with both the extent and activity.[11] It was proved that serum IgG antibodies from vitiligo patients, but not from healthy controls, are capable of penetrating into cultured melanocytes in vitro, and triggering them to engage in apoptosis.[13]

To the best of our knowledge, no study has been published to determine the role of the MIG in the pathogenesis of vitiligo. In this study, serum MIG level in vitiligo patients was measured in a trial to explain its possible role in the pathogenesis of the disease. The current study results revealed that serum MIG level, were significantly increased in vitiligo patients compared to control group. Its level also showed a positive correlation with disease activity as detected by VIDA score. This suggests that elevated serum MIG level could play a role in the pathogenesis vitiligo.

It was suggested that the MIG has a role in maximizing antibody production as both the MIG and CXCR3 may be important for optimizing T cell/APC/B cell interactions and thereby, B cell function including both calcium signals and chemotaxis in activated B cells and favouring humoral immunity. Moreover, MIG and other CXCR3 ligands [interferon inducible protein-10 (IP-10) and IFN-inducible T cell α-chemoattractant-CXC chemokine ligand-11 (1-TAC)] may also influence antibody levels by affecting the migration of activated/memory B cells outside of lymphoid tissue, e. g., to peripheral sites where interactions with stromal cells can support optimal B lymphoblast survival and differentiation.[14] This could explain the positive correlation between serum MIG level and VIDA score of vitiligo in the current study.

Recently, Rashighi et al. have performed in vitro study and revealed a 50-fold induction of the interferon gamma (IFN-γ)-dependent chemokines, the MIG, and IP-10 in vitiligo-affected skin more than in non-lesional skin. Additionally, CXCR3, their common receptor, is expressed on melanocyte-specific CD8+ T cells in the blood. This revealed a critical role for the IFN-γ-chemokine axis in the pathogenesis of vitiligo and supported a strategy of targeting this axis for the development of new drugs.[15]

Since the serum MIG levels are elevated in various autoimmune diseases,[3,4,5,16] the MIG is considered an important driving factor for T cell autoimmunity.[1,14] Studies have demonstrated the presence of skin-homing melanocyte-specific cytotoxic T lymphocytes (CD8+ T cells) in the peripheral blood of patients with vitiligo.[17,18] These T cells are mainly melanocyte-reactive CD8+ T cells and can destroy skin melanocytes with subsequent depigmentation in vitiligo.[7] The frequency of these lymphocytes correlates with both the extent and activity of the disease.[17,18]

Keratinocytes may contribute to this disease process by presenting melanocyte antigens in a MHC class II restricted manner after phagocytosis of melanosome, the presentation of antigen associated with MHC class II to CD4+T cells leads to its stimulation to secrete Th1 cytokines (IL2 and IFN-γ).[19] The MIG is mainly induced by IFN-γ. It binds to CXCR3 receptor expressed by Th1 cells stimulating T cell recruitment and proliferation to promote Th1 cell survival.[1,15] Local production of MIG in vitiligo may maintain keratinocyte MHC II expression, thereby contribute to activity of the disease.

CONCLUSION

Serum MIG could be implicated as an important cytokine in the pathogenesis of vitiligo because its serum level is significantly elevated in vitiligo. Serum MIG level is correlated with the diseases activity suggesting that it could be a used as a marker for assessing vitiligo activity and may open the way for further therapeutic approaches for vitiligo. To the best of our knowledge, this is the first work to study the possible role of the serum MIG in the pathogenesis of vitiligo.

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