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Assessment of serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) among non-segmental vitiligo patients: a pilot study

Azmy Ahmed Abdellatif[1], Amr Mohamed Zaki[1], Hamed Mohamed Abdo[1], Dalia Gamal Aly[2] ✉, Tarek Ahmed Emara[2], Safinaz El-toukhy[3], Hanaa Mohamed Emam[2], Mahetab Samir Abdelwahab[2]

Abstract

Introduction: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is an essential factor in the growth and maturation of blood cells as well as modulation of the immune system. Few studies have investigated its involvement in the development of vitiligo, and no studies have been performed on Egyptian patients.
Aim: To assess GM-CSF serum level among non-segmental Egyptian vitiligo patients and to determine its possible role in the etiopathogenesis of the disease.
Methods: Forty patients with non-segmental vitiligo and 40 age- and sex-matched subjects were assessed for levels of GM-CSF in serum using the ELISA technique.
Results: The patients in this study showed lower levels of GM-CSF in serum compared to controls (mean ± SD was 33.4 ± 5.7 pg/ml versus 63.4 ± 7.4 pg/ml, respectively, $p = 0.0001$). No appreciable relation was detected between levels of GM-CSF in serum and age, sex, family history, and stressful events or disease activity, type, and extent, $p > 0.05$.
Conclusions: GM-CSF serum level may be one of the determinants of the autoimmune hypothesis in the etiopathogenesis of non-segmental vitiligo.

Keywords: GM-CSF, non-segmental vitiligo, Egyptian patients

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Introduction

Vitiligo is a chronic disorder that affects a large number of people all over the world. Genetic factors and several related genes are considered to play an important role in its development (1). Multiple theories have been proposed for its development, including the hydrogen peroxide theory, the cytotoxic metabolites theory, the neural theory, the growth factor theory, and the melanocytorrhagy theory. Animal models and case reports also support the hypothesis that viral infections may play a role in the disease. However, none of these mechanisms are decisively proven (2).

Several studies have demonstrated strong support for the autoimmune theory, which proposes that the loss of melanocytes could arise via the destruction of pigment cells by the immune system. The occurrence of vitiligo with Addison's disease, alopecia areata, pernicious anemia, and Hashimoto's thyroiditis also favors the autoimmune hypothesis of the disease (3).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is part of the family of hematopoietic cytokines. It is released by a range of cell types, including endothelial cells, activated T-cells, monocytes, macrophages, mitogen-stimulated B-cells, and fibroblasts in the form of a single-stranded glycoprotein that has 128 amino acids with a covalent bond (4). Granulocyte-macrophage colony-stimulating factor can stimulate stem cells to develop into various types of mature blood cells and has been primarily found to cause bone-marrow precursor cells to produce both macrophages and granulocyte colonies. It also arbitrates important functions in antitumor immune reaction and in host response to external stimuli. These crucial roles result from its ability to influence the function of mature and immature myeloid cells, such as eosinophils, macrophages, dendritic cells (DCs), and granulocytes (5).

Recent studies indicate that GM-CSF plays a central role in the pathogenesis of several autoimmune and inflammatory diseases, including multiple sclerosis, rheumatoid arthritis, and autoimmune and hereditary pulmonary alveolar proteinosis. It has been reported that its overexpression in the stomach can lead to autoimmune gastritis. Moreover, increased levels of GM-CSF auto-antibodies have also been found in patients with Crohn's disease (6).

The role of GM-CSF in autoimmune and inflammatory disorders makes it of interest for assessment in vitiligo. The data for this role comprise worsening disease in animals by targeting the GM-CSF gene or by blocking the GM-CSF antibody (7).

To the best of our knowledge, few studies have considered the role of GM-CSF in the pathogenesis of vitiligo (8, 9), with no studies performed on Egyptian patients. Therefore, the aim of this work was to assess GM-CSF levels in the serum of Egyptian patients with non-segmental vitiligo.

Patients and methods

Patients

This pilot study included 40 patients (25 females and 15 males) with non-segmental vitiligo. Patients were sub-classified into 20 patients with active vitiligo and 20 patients with stable disease. Forty volunteers served as controls (27 females and 13 males) and had the same age and sex as the patients. The clinical diagnosis was supported by the existence of well-demarcated, depigmented

[1]Department of Dermatology, Venereology, and Andrology, Al-Azhar University, Cairo, Egypt. [2]Department of Dermatology and Venereology, National Research Center, Giza, Egypt. [3]Department of Medical Biochemistry, National Research Center, Giza, Egypt. ✉**Corresponding author: dalia.g.aly@gmail.com**

patches, confirmed by Wood's lamp examination. We excluded patients receiving topical treatment for the previous 2 weeks or systemic treatment for the previous 2 months prior to the study, and those with any associated autoimmune or systemic disease. All patients and controls were selected from the National Research Center's dermatology clinic. All subjects gave informed consent to participate in this study. The work was approved by the research ethics board at the National Research Center in Giza, Egypt.

Methods

A complete history was taken from all subjects, followed by a clinical examination and measurement of GM-CSF levels in sera. The activity of vitiligo was defined based on the evolution of previously affected areas or the emergence of novel areas in the previous 3 months (10) and inactive disease was classified based on the lack of evolution of previously affected areas or emergence of new areas in the previous 6 months (11).

The extent of vitiligo was assessed using the Rule of 9 following Hamzavi et al. (12), which is the approximate percentage of the body surface area involved. Skin phototype was determined according to the Fitzpatrick Scale, which is a numerical classification scheme for skin color (13).

Assessment of GM-CSF serum levels

Measurement of the GM-CSF levels in the sera of all patients and controls was carried out after drawing a 3 ml blood sample from each of them. Centrifugation of the samples was performed followed by freezing the sera, which was kept at –20 °C until assessment. GM-CSF assessment was performed by means of the Enzyme-Linked Immunosorbent Assay Human GM-CSF kit, LabsTM Inc. Biotechnology, Canada. The investigational methodologies were carried out based on the information supplied by the company.

Calculation of results

To calculate the concentration of patients' samples, the negative control absorbance was deducted from the observed one. Then the optical density of each standard was plotted against its concentration (pg/ml) using a logarithmic scale to construct the "standard curve." The equivalent concentration of Human GM-CSF in pg/ml in patients' samples was determined by plotting the subtracted absorbance value of all samples on the standard curve.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 18 for windows SPSS; Inc, Chicago, IL was used for data analysis. Continuous data were expressed as mean and standard deviation. Number and percent were used to describe categorical information. A t-test was used for comparing between two means and a chi-square test for comparing between two qualitative variables. To correlate between two continuous variables, the Pearson correlation test was used. $P < 0.05$ was considered statistically significant.

Results

Out of the 40 patients with non-segmental vitiligo enrolled in this study, 25 were females (62.5%) and 15 were males (37.5%). Their age ranged from 10 to 71 years with a mean ± SD of 31.1 ± 17.3 years. The control group comprised 27 females (67.5%) and 13 males (32.5%). Their age varied from 13 to 72 years with a mean of 30.50 ± 17.5 years. There was no statistical distinction between patients and controls regarding age and sex ($p > 0.05$).

Table 1 | Comparison between patients and controls for serum levels of granulocyte-macrophage colony-stimulating factor.

Variables	Patients (n = 40)	Controls (n = 40)	*P*
GMCSF (pg/ml)			
mean ± SD	33.4 ± 5.7	63.4 ± 7.4	0.0001*

*Significant

Among the 40 patients, 20 (50%) patients had active vitiligo and 20 (50%) had stable disease. Family history of vitiligo was positive in 10 (25%) of the patients. Stress was reported by 25 (62.5%) patients to be an aggravating factor for the disease. Clinical assessment of the patients revealed that 32 patients (80%) had generalized vitiligo, seven (17.5%) had acrofacial vitiligo, and only one (2.5%) had focal vitiligo. Skin phototype was divided into five categories: five (12.5%) patients had Type 2 skin phototype, 12 (30%) had Type 3, 21 (52.5%) had Type 4, and two (5%) had Type 5.

On comparing the patients to the control group by serum levels of GM-CSF, we noted considerably lower GM-CSF levels in the sera of vitiligo patients; the mean ± SD was 33.4 ± 5.7 pg/ml versus 63.4 ± 7.4 pg/ml, respectively, $p = 0.0001$ (Table 1).

No statistically significant difference was noted when comparing the GM-CSF levels in the sera of patients with various variables such as age, sex, family history, stress, disease activity, and type, $p > 0.05$ (Table 2).

Moreover, no noteworthy association was detected between GM-CSF levels in the sera of patients for either skin phototype or disease extent ($r = 0.1$, –0.2, respectively, $p > 0.05$).

Discussion

A limited number of studies, in different populations, have been performed in an attempt to understand the mode of action of GM-CSF in vitiligo, but with conflicting results (9, 15, 16) because the GM-CSF levels in either sera or lesional vitilgo skin was quite variable. Low levels of GM-CSF have been recognized circulating in the sera of individuals that rise in inflammatory diseases or immune reactions (15). Nevertheless, in the current study we observed a decreased GM-CSF serum level in Egyptian patients with non-segmental vitiligo compared to their age- and sex-matched controls. Human melanocytes have receptors for GM-CSF (18, 19),

Table 2 | Granulocyte-macrophage colony-stimulating factor serum level by patient variables.

Variables	Granulocyte-macrophage colony-stimulating factor (mean ± SD)	*P*
Age (years)		
10–40	32.4 ± 5.6	0.08
› 40	36.0 ± 5.5	
Sex		
Male	34.5 ± 7.5	0.4
Female	32.8 ± 4.4	
Family history		
Negative	34.0 ± 6.3	0.3
Positive	31.8 ± 3.3	
Stress		
Negative	32.3 ± 5.1	0.3
Positive	34.1 ± 6.1	
Vitiligo activity		
Active	32.4 ± 6.5	0.3
Stable	34.4 ± 4.8	
Vitiligo type		
Generalized	32.9 ± 5.7	0.5
Acrofacial	34.6 ± 5.7	

whereby GM-CSF can work as a mitogenic stimulator on them, indicating that its deficiency may play a role in the depigmentation process in the disease (20).

Few reports were in agreement with our findings, such as that by Moretti et al. (16), who demonstrated increased tumor necrosis factor (TNF)-α and interleukin (IL)-6 and decreased GM-CSF and basic fibroblast growth factor (BF-GF) in lesional vitiligo skin. Martinez-Esparza et al. (17) also showed a decrease of GM-CSF in lesional vitiligo lesions. Moreover, Yu et al. (8) noted that vitiligo patients with active disease had a reduction in the formation of GM-CSF via mononuclear cells.

There is increasing proof that cytokines play a vital function in the autoimmune process occurring in vitiligo, explaining the depigmentation process taking place in the disease. Our findings together with those of the previous studies point to an imbalance in cytokine levels in vitiligo, which could impair the normal lifespan and function of melanocytes and thus recovery from vitiligo. Moretti et al. (16) found increased TNF-α and IL-6, which are paracrine inhibitors of melanocytes, and decreased GM-CSF and BF-GF, which have a stimulating effect on melanocytes, which could be linked to this hypothesis. It should be noted that the previous studies were carried out on vitilignous skin whereas our work was performed on serum. We believe that the correlation of serum cytokine levels with the epidermal cytokine microenvironment needs to be explained in greater detail.

Interestingly, Campbell et al. (21) demonstrated that mice deficient in GM-CSF were found to have a noticeable decrease in the frequency and pathology of collagen-induced arthritis. This contrasted with our results because our patients with vitiligo (whether active or stable disease) had low GM-CSF serum levels compared to controls, indicating that its reduction helped in the initiation and/or progression of the disease.

Conversely, Tu et al. (9) noted that the sera of vitiligo patients with either the generalized or focal subtypes showed an increase in GM-CSF levels. In addition, patients with active vitiligo exhibited raised levels of GM-CSF serum compared to patients with inactive disease, suggesting that GM-CSF could play a role in the development of vitiligo.

Determining whether or not raised GM-CSF levels play a role in triggering the autoimmune process in vitiligo needs to be evaluated. The exact explanation for the partially overlapping results regarding the formation of GM-CSF in the disease and the mechanisms behind its role in vitiligo is not clearly known. Does its in vitro role differ from in vivo, and from one autoimmune disorder to another, or even in the same disorder? Can its increase as well as decrease be related to the pathogenesis of vitiligo, and can this be a part of multiple factors such as the family history, which was quite high in our study? This remains to be evaluated.

We believe that the confined presence of GM-CSF could be sufficient to modify tolerance and trigger an autoimmune reaction by T-helper cells via activation of DCs. Dendritic cells may exert their tolerogenic roles via the production of regulatory cells (Tregs) which are activated by tolerogenic DCs (22). It is probable that GM-CSF activates Tregs through a diverse method and that the development of these cells directly affects the DCs phenotype and function. This could be in agreement with the idea that T cells ought to be resistant to low levels of GM-CSF so as to prevent an exaggerated response to the low levels of GM-CSF produced by the innate immune system (23). To conclude, GM-CSF may be one of the determinants of the autoimmune hypothesis claimed in the etiopathogenesis of non-segmental vitiligo. Future larger-scale studies are warranted to confirm our findings.

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