Dermatology Practical & Conceptual

Evaluation of IL-38, a Newly-introduced Cytokine, in Sera of Vitiligo Patients and Its Relation to Clinical Features

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ABSTRACT

Introduction: Vitiligo is thought to be an autoimmune disorder caused by melanocytes dysfunction and depigmentation. Among different executors of the immune system in developing the disease, the role of various cytokines has been defined.

Objectives: We have focused on IL-38, the tenth member of IL-1 cytokine family with a proposed anti-inflammatory role, which has not hitherto been introduced as an anti-inflammatory factor in vitiligo.

Methods: Sixty-nine generalized vitiligo patients and 72-year-old- and sex-matched healthy individuals were included in this study. IL-38 level was evaluated in sera of all participants using ELISA assay. The relation of IL-38 level to patients characteristics was evaluated.

Results: IL-38 serum level in vitiligo patients (159.5±39.7 pg/ml) was lower than the healthy controls (166.7±34.8pg/ml) (P = 0.039). A weak negative correlation between the age of male patients and their IL-38 serum levels was identified (r = 0.38, P = 0.058). Evaluation of the IL-38 serum levels relationship with patients clinical characteristics showed no correlation with disease onset, stage of depigmentation, and disease activity status.

Conclusions: The lower levels of IL-38 as an anti-inflammatory cytokine support the inflammatory nature of vitiligo. It indicates the difference of IL-38 in sera of vitiligo patients and healthy controls, as the first report of the lower level of this cytokine in the context of vitiligo. The reason of this difference remains to be clarified; as there are not sufficient study reports revealing the role of gender, ethnicity and inflammation on the cytokine network in the context of vitiligo.

Introduction

Vitiligo is an acquired and progressive disorder, caused by melanocytes dysfunction and chronic depigmentation, resulting in the formation of demarcated white macules in the epidermis [1]. The prevalence of vitiligo estimated to be in the range of 0.4% to 2.0 % worldwide [2] with a reported greater predilection to arise in childhood and based on some studies in females [1, 3]. This disease is classified into three main clinical types according to international consensus; namely, non-segmental or generalized vitiligo, segmental vitiligo and mixed vitiligo [4]. These subtypes are distinguished based on their onset and distribution patterns [4]. The mechanisms underlying the etiopathogenesis of vitiligo are not clearly identified; however, in genetically susceptible individuals, several phenomena have been supposed to be associated with the disease onset and development. Autoimmunity, oxidative stress, impairment of melanocytes growth and development, viral infections and neurological damages are among the main theories proposed in the etiology of vitiligo [4]. Autoimmune theory implicates the remarkable presence of immune system footprints in the context of the disease, as high serum levels of autoantibodies against tyrosine hydroxylase (TH) which cause the melanocytes and keratinocytes destruction are detectable in vitiligo patients [5]. Moreover, it has been found that skin-homing cytotoxic T-cells specific for some peptides of melanocytic proteins gp-100 and melan A or melanoma antigen recognized by T cells (MART)-1, are related to vitiligo disease activity [6]. Also, a significant rise in serum levels of cytokines such as tumor necrosis factor (TNF)-α , interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-6, IL-17, IL-22, IL-8 and IL-10 was observed in these patients [7-11].

IL-38, with the previous name, IL-1HY2 is a member of IL-1 cytokine family (IL-1α, IL-1β, IL-1 receptor antagonist [IL-1Ra], IL-18, IL-36 receptor antagonist [IL-36Ra], IL-36α, IL-37, IL-36β, IL-36γ, IL-38 and IL-33) [12]. This cytokine is expressed in a variety of organs and tissues including heart, placenta, skin, spleen, thymus, fetal liver and proliferating B cells of the tonsil [12]. The genomic position of IL-38 is near the IL-1Ra and IL-36Ra locus on human chromosome 2p13 [13]. Furthermore, the structural homology of IL-38 with IL-1Ra and IL-36Ra is about 41% and 43%, respectively, whereas this homology is significantly lower (14%-30%) with the other members of IL-1 cytokine family [14]. Because of its structural resemblance with IL-36Ra, IL-38 is supposed to function through IL-36R downstream signaling pathways such as nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) inhibition [15,16]. In line with these findings about the biological functions of IL-38, several studies have focused on the specific role of this cytokine in some disorders, typically immune-mediated diseases. IL-38 gene polymorphisms have been studied in ankylosing spondylitis, rheumatoid arthritis (RA), psoriatic arthritis and heart diseases [17-20]. Moreover, the levels of this cytokine have been raised in sera of patients with systemic lupus erythematosus (SLE), RA, myocardial infarction and childhood asthma [21-24].

Objectives

According to the role of various cytokines in the pathogenesis of vitiligo, focusing on those with the unknown effects in this disease would come into consideration. Therefore, we aimed to investigate the role of IL-38 in vitiligo as the first report of the lower level of this cytokine in the context of vitiligo pathogenesis.

Methods

Subjects

A total of 76 patients with vitiligo (69 generalized and 7 localized) who referred to Dermatology Outpatient Clinic of Shahid Faghihi Hospital, Shiraz, Iran, were included in this study. Of 69 patients with generalized vitiligo, 43 and 26 patients were females and males, respectively (mean age 36.00±14.88 years; range 13-74 years). The study protocol was approved by Elite Researcher Grant Committee under award number [971223] from the National Institute for Medical Research Development (NIMAD), Tehran. Informed consent was obtained from all patients. Information from the patients was recorded using a special questioner with respect to demographic, paraclinical and clinical presentation of the cases. Subjects with other autoimmune or inflammatory diseases, current infections and pregnant women were excluded from the study.

Vitiligo disease activity score (VIDA) was used to evaluate vitiligo activity [25]. In addition, according to the Vitiligo European Task Force (VETF) system, the extent, stage (cutaneous and hair pigmentation in vitiligo patches) and progression of the disease was determined for each patient. The patients were classified into progressive, stable or regressive according to the spreading of the lesions or white macules at the time of blood sampling [26]. Skin type of the patients was also determined according to Fitzpatrick classification [27]. Seventy-two age- and sex-matched healthy subjects (42 females and 30 males; mean age 39.54±10.35 years; range 24-62 years) were considered as the control group. Controls were healthy individuals who had no history of autoimmune disease themselves or in their relatives.

Sample preparation and cytokine measurement through enzyme-linked immunosorbent assay (ELISA)

Three milliliters of blood were taken from each patients and controls. In order to isolate sera, the samples were centrifuged at 4000 rpm for 15 minutes. Sera were stored at -70ºC until used.

Serum level of IL-38 in all individuals was measured through a specific ELISA kit (R&D SystemsCat. No. DY9110-05) according to the manufacturer instructions. Briefly, anti-IL-38 monoclonal antibodies were pre-coated on to ELISA microplates. Serum samples (100 µl) were added to each well. Afterwards, 100 µl of the working solution of streptavidin-horseradish peroxidase (HRP) conjugated antibodies were added and after addition of substrate the optical density of each well was determined using a microplate reader (Biotech) at 450 nm.

Statistical Analysis

Statistical analysis was performed using SPSS v. 25 software (SPSS, Inc.) and GraphPad Prism v.8 software. Data were presented as mean ± standard deviation (SD). Mann-Whitney U test was used to evaluate IL-38 serum level differences between the groups. Spearman correlation test (rho) was done to assess any correlation between two quantitative variables with no normal distribution. $P < 0.05$ was regarded as statistically significant value.

Results

Demographic and Clinical Data

The participant clinical and demographic data are summarized in Table 1. A total of 76 patients were included in this study. Among them, 69 cases presented with generalized and 7 cases with localized type of vitiligo. Comparison of IL-38 serum levels in generalized (159.53±39.66 pg/ml) and localized (158.99±31.42 pg/ml) patients showed no significant differences. Due to limited number of the localized patients, the rest of analysis was performed only on generalized patients. The beginning of the disease was around 27 years old. Family history of vitiligo was seen in 26.2% of the patients. More than 95% of the patients had generalized bilateral symmetrical vitiligo. Most patients (85.5%) had complete depigmentation with less than 30% hair whitening, and 86.8% were categorized in type III skin type. The disease was progressive in 25 (36.2%) patients, stable in 20 (29%) cases and regressive in 24 patients (34.8 %).

IL-38 Serum Level Differences Between Patients and Healthy Controls

We found a significant difference in IL-38 serum levels between patients (159.5±39.7 pg/ml) with range of 122.1-331.6 pg/ml and healthy control group (166.7±34.8 pg/ml) with range of 113.1-293.0 pg/ml ($P = 0.039$) (Figure 1).

We did not find any significant difference in IL-38 levels between the female (163.8±46.5 pg/ml) and male (152.5±23.6 pg/ml) patients ($P = 0.31$) or female (169.5±40.4 pg/ml) and male (162.8±24.3 pg/ml) controls ($P = 0.92$).

Correlation Between IL-38 Serum Levels and Patients Characteristics

IL-38 serum levels showed no correlation with the age of patients, however when patients were evaluated according to the sex, IL-38 serum levels showed a weak negative correlation with the age of male patients ($P = 0.058$, $r = -0.38$) (Figure 2). This correlation was not observed in relation to male healthy controls ($P = 0.21$, $r = -0.233$).

As the next step, analysis of the relation of IL-38 serum levels to other patients characteristics showed no significant correlation with disease onset, disease duration. And the Comparison of IL-38 serum levels in patients with positive family history of vitiligo and patients with negative family history showed no significant differences. We did not find any difference in IL-38 level in patients with generalized bilateral symmetrical vitiligo (160.32±40.38 pg/ml) compared to other types (142.23±4.13 pg/ml, $P = 0.41$). Stage of depigmentation also showed no significant effect on IL-38 level ($P = 0.85$). IL-38 did not differ between patients with progressive (148.83±21.50 pg/ml), stable (160.90±44.79 pg/ml) or regressive (169.54±47.75 pg/ml) disease, $P = 0.23$). VIDA score had no effects on the level of cytokine. However, evaluation of the relationship between skin type and IL-38 serum levels, showed significant differences in both male and female patients. As, comparison of IL-38 serum levels between two skin type groups of male patients, ie type III (24 patients) and type IV (2 patients) resulted in the evidence of a difference between these two groups ($P = 0.045$) (Figure 3). The level of this cytokine was lower in male patients with type III (149.4±21.7 pg/ml) than those with type IV (190.2±5.45 pg/ml). We compared the IL-38 serum levels

Table 1. Demographic and clinical data of the vitiligo patients.

Characteristics	Patients with generalized vitiligo	Healthy controls
Number of subjects	69	72
Age (year), mean±SD	36.00±14.88	39.54±10.35
Gender (Female/Male), N(%)	43/26 (62.3/37.7)	42/30 (58.3/41.7)
Disease onset (year)), mean±SD	27.01±15.76	
Disease duration (year)), mean±SD	8.98±7.93	
Family history of vitiligo, N(%)	18 (26.1)	
Family history of other Autoimmune disease, N(%)	5 (7.2)	
Smoking, N(%)	17 (24.6)	
Generalized type, N(%): Acrofacial Vulgaris Mixed	 2 (2.9) 66 (95.7) 1 (1.4)	
Stage of depigmentation, N(%) -0 (N1) -Incomplete -Complete with <30%hair whitening -Complete with >30%hair whitening	 2 (2.9 %) 6 (8.7 %) 59 (85.5 %) 2 (2.9 %)	
Skin type, N(%) -I -II -III -IV -V -VI	 - 2 (2.9 %) 60 (87.0 %) 7 (10.1 %) - -	
Spreading, N(%) -Progressive -Stable -Regressive	 25 (36.2 %) 20 (29.0 %) 24 (34.8 %)	
Vitiligo disease activity score (VIDA), N(%) +4 activity of 6 weeks or less +3 activity of 6 weeks to 3 months +2 activity of 3-6 months +1 activity of 6-12 months 0: stable for 1 year -1: Stable at least 1 year with spontaneous repigmentation	 13 (19.1 %) 14 (20.6 %) 12 (17.6 %) 14 (20.6 %) 15 (22.1 %) -	

SD = standard deviation.

among female skin type groups. We could find a IL-38 serum level difference between two groups of female patients, those 36 patients with skin type III (168.29±49.43 pg/ml) and 5 patients with type IV (135.97±9.68 pg/ml, P = 0.018) (Figure 3). Regardless of the differences in IL-38 level observed between male and female patients with type IV and III due to quite low number of type IV patients, statistically, no correlation has been detected, in patients with skin type III, IL-38 levels in male patients (149.4±21.73 pg/ml) were less than in female patients (168.29±49.43 pg/ml) (P = 0.046).

The IL-38 serum level did not differ among patients with various treatments. Patients under phototherapy (154±26.2 pg/ml), phototherapy along with topical steroids (162.9±46.1 pg/ml), and only topical steroids (149.7±20.9 pg/ml).

Conclusions

Interleukin (IL)-38, the tenth member of IL-1 cytokine family, was discovered in 2001 [12]. Because of its sequential homology with IL-1Ra and IL-36Ra, IL-38 has been supposed to exert anti-inflammatory properties [14]. Various studies have focused on the investigation of IL-38 exact role in different disorders particularly autoimmune diseases. Gou et al have shown that IL-38-related polymorphisms

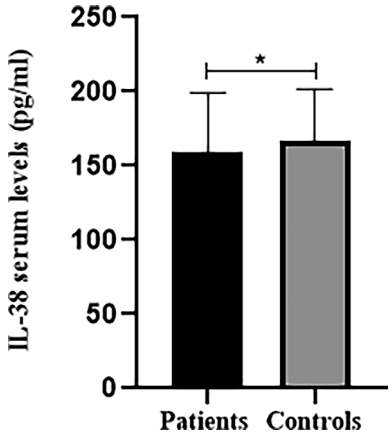

Figure 1. IL-38 serum level differences between generalized vitiligo patients and healthy controls; *P<0.05. Data are represented as mean± standard deviation.

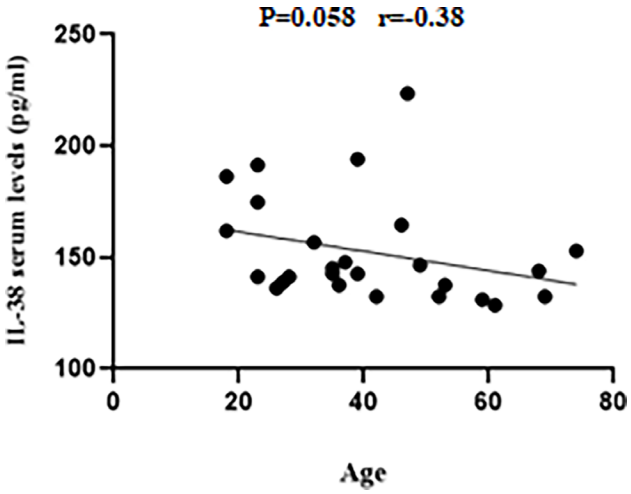

Figure 2. Correlation between the age of vitiligo male patients and their IL-38 serum levels; P = 0.058, r = -0.38

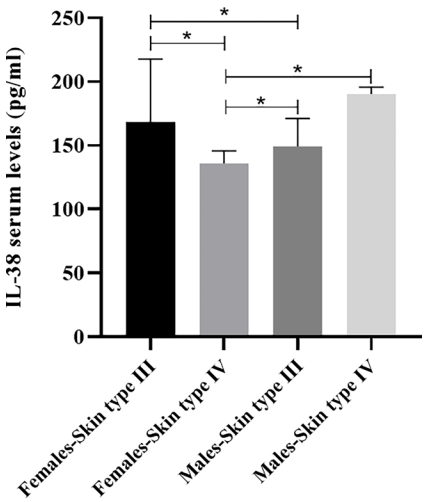

Figure 3. IL-38 serum level differences between female and male patients with skin types III and IV. *: P < 0.05. Data are represented as mean ± standard deviation.

are associated with the increased risk of ankylosing spondylitis [17]. Moreover, an association of IL-38 rs7570267 and rs3811058 single nucleotide polymorphism with RA susceptibility has been shown [18]. In another study on RA patients, elevated levels of IL-38 was reported in plasma of patients compared to healthy controls [28]. Rudloff et al measured the IL-38 serum level in patients with SLE. They found that IL-38 may play a protective role in this disorder [21]. However, the probable role of this newly identified cytokine in other autoimmune disorders has not been clarified yet. Accordingly, we have focused on vitiligo, a disease with an auto-immune background and no published report on the anti-inflammatory role of IL-38 in its pathogenesis. We measured IL-38 level in a group of patients with vitiligo and healthy subjects to evaluate the probable role of IL-38 in disease development. The results showed that the cytokine level in patients was significantly lower than the healthy individuals. It should be noted that. This result was in contrary to the result from previous study where Radwa El- Sayed et al measured the IL-38 serum level in 21 patients with vitiligo and 21 healthy controls. They found that IL-38 serum level was higher in patients with vitiligo than in healthy controls and was related to vitiligo severity and signs of activity [29]. The reasons for these differences may be due to sample size and genetic background, and also our result was different with the results reported from some other studies in RA [28] and SLE [21] patients. The reason for these differences is not exactly clear but may be due to variation in the biological nature of the diseases as type and the extent of inflammation established in various disorders may be different. In this regard, we could state the increased serum levels of autoantibodies such as anti-dsDNA, anti-Ro, antinuclear antibody (ANA) and low serum levels of complement components, ie C3 and C4 and high serum erythrocyte sedimentation rate (ESR) as the indicators of inflammatory condition in SLE, whereas these factors are not considerable in the context of vitiligo. Moreover, the contribution of genetic background and sample size should not be ignored as these are also important factors that might influence such differences.

It should be noted that, the current finding is similar to our previous study about the role of IL-38 in Bechet disease (unpublished data) in which a significant lower IL-38 levels in patients compared to controls was found. In addition, in various studies the elevated levels of several inflammatory cytokines such as IL-1β, IL-8, IL-17 and IFN-γ in sera of vitiligo patients compared to healthy controls have been shown [7-10]. According to these data vitiligo is proposed as an auto-inflammatory disease. Thus, lower levels of IL-38 which seems to be an anti-inflammatory cytokine, in these patients would be expected and support the inflammatory nature of this disease.

On evaluation of the relation of IL-38 level to patients characteristics, we found a weak negative correlation

between the age of male patients and their IL-38serum levels, so that this cytokine showed a decreased level with increased age of the patients. This correlation was not found in female patients as well as male and female controls.

We investigated the relation of IL-38 level to patients disease activity and found no significant result. Analysis of IL-38 level in patients with various stages of depigmentation and VIDA score did not also reach to be significant. These data may suggest that IL-38 have no important role in the pathogenesis and activity of vitiligo.

Collectively, we could indicate the difference of IL-38 in sera of vitiligo patients and healthy controls, as the first report of the lower level of this cytokine in the context of vitiligo. The reason of this difference remains to be clarified; as there are not sufficient study reports revealing the role of gender, ethnicity and inflammation on the cytokine network in the context of vitiligo.

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