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T helper and regulatory T cell cytokine profile in active, stable and narrow band ultraviolet B treated generalized vitiligo

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ABSTRACT

Background: Vitiligo is a T cell mediated autoimmune depigmenting disease. Altered cytokine concentrations have been suggested in the pathogenesis of vitiligo.
Methods: T helper and regulatory T cell cytokines (IL-2, IFN-γ, IL-10, IL-13, IL-17 and TGF-β) have been estimated by ELISA and their clinical correlation was determined. The study had 3 groups: group I with 80 vitiligo patients (60 active and 20 stable), group II with 25 narrow band ultraviolet B treated vitiligo and group III with 70 healthy controls.
Results: Significant difference was found in the serum interleukin (IL)-10, IL-13, IL-17A and TGF-β1 concentrations among 3 groups ($P < 0.05$). In group I, serum IL-2, IL-17A concentrations were significantly increased and TGF-β1 concentrations were decreased in active vitiligo compared to stable vitiligo ($P < 0.05$). Concentrations of IL-2, IL-10 and IL-13 (rho = −0.307, rho = −0.407, rho = −0.351 and $P < 0.05$; respectively) were negatively- and TGF-β1 concentrations were positively-correlated (rho = 0.799, $P = 0.001$) with disease duration. Interleukin-13 concentrations were negatively- and serum TGF-β1 concentrations were positively-correlated (rho = −0.326, rho = 0.244 and $P < 0.05$; respectively) with percentage of body surface area involvement.
Conclusions: Increased concentrations of serum IL-10, IL-13, and IL-17A and decreased concentrations of TGF-β1 suggested altered cell-mediated immunity that may facilitate the melanocyte cytotoxicity in vitiligo.

1. Introduction

Vitiligo is an acquired skin depigmentation disorder characterized by the patchy loss of functional melanocytes from the epidermis. The precise etiology of vitiligo is not known and there is no consensus about the pathogenesis of vitiligo. There are three major hypotheses namely, (a) biochemical [1], (b) neural [2,3], and (c) autoimmune [4,5]. Other possible contributing factors such as (i) complex biochemical imbalance due to defective free-radical defense interfering with melanin content and synthesis [6–8], (ii) deficiency in melanocyte growth factors [9], (vi) defect of melanocyte adhesion [10] or (vii) genetic factors [11] have been put forward in the pathogenesis of vitiligo. But the most compelling is the autoimmune hypothesis applied to generalized vitiligo. Altered CD4+ T cell function and autoreactive melanocyte specific cytotoxic T cells have been reported in the pathogenesis of vitiligo supporting autoimmune hypothesis [5,12–16]. Moreover, alteration in the specialized subpopulation of CD4+ T cells — T helper 1 (Th1), Th2, Th17 and regulatory T cells (Tregs) has been implicated in autoimmune disorders and same has been suggested in vitiligo [5,12–20]. Th1 cells predominantly produce interferon (IFN)-γ, tumor necrosis factor (TNF)-β and interleukin (IL)-2; Th2 cells produce IL-4, IL-5, IL-10, and IL-13; Th17 cells produce IL-17, and IL-22 and Tregs synthesize TGF-β [19,20]. Although, the major cytokines produced by T cell sub-populations have been studied in vitiligo but the role of cytokines in the pathogenesis of vitiligo has not been completely understood. Previous studies of serum cytokine concentrations in vitiligo are few and results are often contradictory. Further, narrow-band ultraviolet B radiation (NB-UVB) therapy offers one of the most effective treatment modality for vitiligo but the mechanism of action of NB-UVB is not well understood. The NB-UVB therapy induces perifollicular pigmentation suggesting that it influence melanocyte reserve in the outer root sheath of hair follicle [21]. However, a two step effect of NB-UVB has been suggested but may also occur simultaneously [22]. Firstly, there is a local or systemic immuno-modulation leading to downregulation of immune attack against the melanocytes and subsequent stimulation of melanocytes to migrate to epidermis and synthesize melanin [5]. The effect of NB-UVB on T helper and Treg cytokines in active vitiligo has not been studied and present study is the only to report the findings. Thus, the purpose of this study was to reveal the status of Th1,

Abbreviations: IL, interleukin; IFN, interferon; Th, T helper; Treg, regulatory T cell; NB-UVB, narrowband ultra violet B; GV, generalized vitiligo; TGF, transforming growth factor; TNF, tumor necrosis factor.

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Th2, Th17 and Treg cytokines in generalized vitiligo (GV), NB-UVB treated GV and their clinical correlations that was lacking in the previous studies.

2. Materials and methods

2.1. Patient and control groups

The present study had 3 groups. Group I had 80 untreated patients with generalized (active n = 60; and stable n = 20) vitiligo (GV). In the present study, GV involves three clinical phenotypes acrofacial, vulgaris and mixed (acrofacial plus vulgaris) type of vitiligo and excluded segmental type of vitiligo. In group I, none of the patients were treated with systemic corticosteroids or other immunosuppressive therapy in the preceding one month of blood collection. Patients with other inflammatory skin disorders and taking any medication (topical or oral) were excluded from the study. Patients with any past or current history of smoking, alcohol and drug usage were also excluded to avoid the influence of such factors. The course of vitiligo was defined as active when there was appearance of new lesion(s) and progression of old lesions within 3 months, and as stable when there was no appearance of new lesion(s) and progression of old lesions within 6 months. The total disease duration was defined as the period when the first depigmented lesion appeared to the day of sample collection during the first time attack of disease (i.e., first episode of disease). Koebner phenomenon positivity was found in eleven patients and seven patients had other associated autoimmune disorders such as autoimmune arthritis, autoimmune thyroiditis, and type I diabetes mellitus. In group II, all patients have received treatment with narrowband ultraviolet B (NB-UVB) to whole body 3 times per week on non-consecutive days according to standard protocol; the initial dose of NB-UVB was 0.28 J/cm^2 with 20% increment of previous dose per next visit and treatment was continued to a maximum dose of 2 J/cm^2. The sample was collected one week after the final dose. In this group, we enrolled n = 51 patients, but only n = 25 patients completed the narrowband ultraviolet B treatment (initial dose = 0.28 J/cm^2 and final dose = 2.5 J/cm^2) as per our standard protocol. The remaining patients discontinued the treatment and hence were excluded from the study. Patients with any past or current history of smoking, alcohol and drug usage and other associated inflammatory skin diseases were also excluded in this group. The present study has shown the data for only n = 25 patients. Koebner phenomenon positivity was found in three patients. In group III, we enrolled n = 70 age- and sex-matched healthy volunteer individuals having no past or current history of any other skin diseases including vitiligo/inflammatory/associated autoimmune diseases. Individuals with any past or current history of smoking, alcohol and drug usage were also excluded in this group. The mean ages in each group were 27.4 ± 1.4, 28.2 ± 1.4 and 30.6 ± 1.3 respectively. Age, sex, total duration of the disease, site and age of onset, percentage of the body surface area involvement, progression of disease, Koebner phenomenon positivity, family history of vitiligo and any associated systemic or autoimmune diseases, were recorded. Written informed consent from vitiligo patients and control subjects, and institutional ethics committee approval were obtained.

2.2. Methods

Serum samples were collected and serum cytokine concentration of IL-2, IFN-γ, IL-10, IL-13, IL-17A and TGF-β1 (Ready-set-go, eBiosciences Inc. San Diego, CA), were detected quantitatively by the enzyme-linked immunosorbent assay (ELISA) method in all three groups and concentration was expressed as pg/ml. ELISA tests were performed according to manufacturer's instructions. Briefly, 96 well ELISA plate (Corning Costar 9018) was coated with capture antibody in coating buffer and incubated overnight at 4 °C followed by washing. Wells were blocked with 1× assay diluent and incubated at room temperature for 1 h followed by washing. Serum samples and diluted standards (2 fold serial dilutions were performed using the top standard (recombinant — IL-2, IFN-γ, IL-10, IL-13, IL-17A and TGF-β1) to make the standard curve) were then incubated overnight at 4 °C to provide maximal sensitivity and washed. Detection antibody was added followed by 1-h incubation and washing. Avidin-horseradish peroxidase was then added followed by incubation and washing. Substrate solution (1× TMB — 3,3′,5,5′-tetramethylbenzidine) was then added followed by 15 min incubation. The reaction was terminated by adding stop solution (2 mol/l sulfuric acid) and plates were read at 450 nm. The concentration was determined against the standard curve. ELISA test was performed in triplicates for each sample to ensure minimal variation between the wells. The standard curve range of kits used were: IFN-γ 4-500 pg/ml; IL-2 4-500 pg/ml; IL-10 2-300 pg/ml; IL-13 4-500 pg/ml; IL-17A 4-500 pg/ml; and TGF-β1 4-500 pg/ml respectively. The serum cytokine concentration of patients with vitiligo were compared among the three groups and correlation of serum cytokine with age, sex, percentage of body surface area involvement, and duration of vitiligo were studied. The instrument used for the estimation of concentration of cytokines was "Bio-Rad iMark" microplate reader and the study was carried at the Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi.

2.3. Statistical analysis

The one way analysis of variance (one way ANOVA) with Bonferroni correction was used to compare means between the groups. In group I, the means of active and stable vitiligo were compared by independent sample *t* test. Spearman's rank correlation was used for relationship studies between quantitative parameters. Logarithmic transformation of the data was applied and data was presented after taking antilogarithm (Table 1). The significance concentration was set at P less than 0.05 and 0.01. Statistical analyses were performed using a software package (Stata, version 9 for Windows, StataCorp LP).

3. Results

Significant difference was found in the mean serum cytokine concentration of interleukin-10, IL-13, IL-17A and TGF-β1 (ANOVA, $P < 0.05$) between the groups but no significant difference was detected for IL-2 and IFN-γ (ANOVA, P = 0.262, P = 0.152, respectively) among the groups (Table 1). Serum IL-10 and IL-13 concentrations were found to be increased in group I (generalized vitiligo, GV) and group II (NB-UVB treated GV) compared to group III (matched controls) but interestingly, IL-13 were higher in group I compared to group II whereas

Table 1
Comparison of mean ± SD of serum cytokine concentration between untreated (group I), narrowband ultraviolet B treated (group II) vitiligo and controls (group III).

Cytokine	Group I Untreated vitiligo	Group II NB-UVB treated vitiligo	Group III Controls	P value
(pg/ml)	(n = 80)	(n = 25)	(n = 70)	
IL-2				
Mean ± SD	10.67 ± 2.02	10.67 ± 1.42	9.03 ± 1.93	NS
IFN-γ				
Mean ± SD	4.57 ± 1.56	4.21 ± 1.61	5.11 ± 1.56	NS
IL-10				
Mean ± SD	7.24 ± 1.63	9.44 ± 1.55	5.71 ± 1.7	0.001
IL-13				
Mean ± SD	8.09 ± 1.80	6.10 ± 1.96	5.03 ± 1.37	0.001
IL-17A				
Mean ± SD	5.99 ± 1.63	5.54 ± 1.5	4.27 ± 1.96	0.001
TGF-β1				
Mean ± SD	1756.85 ± 1.84	3032.67 ± 1.21	3873.83 ± 1.36	0.001

Data represented in mean ± SD and ANOVA (analysis of variance) was used, $P < 0.05$.
IL — interleukin, TGF — transforming growth factor, IFN — interferon, NB-UVB — narrowband ultraviolet B.