Estimate the Serum Level of IL-17A and TGF-β1 1 in Iraqi Generalized Vitiligo Patients

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Abstract

Vitiligo is an acquired idiopathic skin disease characterized by white macules result from the destruction of melanocytes. Several cytokines have been implicated in the pathogenesis of vitiligo, keeping this in view the current study aimed to evaluate the serum levels of IL-17A and TGF-β1 in 25 patients with generalized vitiligo and compared them with healthy individuals in order to investigate whether these cytokines imbalances plays a role in the pathogenesis of this depigmentary disorder. Results of the present study showed that the serum level of IL-17A in vitiligo patients was increased significantly ($p \leq 0.05$) while the serum level of TGF-β1 was decreased significantly ($p \leq 0.05$) as compared with healthy control. According to the gender of patients, males had a slightly non-significant increased ($p<0.05$) in the level of IL-17A and TGF-β1 as compared with females. Patients with early onset vitiligo had non-significant increased ($p<0.05$) in the level of IL-17A and TGF-β1 as compared with late onset vitiligo patients. No significant differences ($p<0.05$) were detected in the levels of IL-17A and TGF-β1 in patients regarding the clinical types of vitiligo, family history and Koebner phenomenon. Patients with active disease had a significant increase ($p \leq 0.05$) in the level of IL-17A, though a significant decrease ($p \leq 0.05$) in the level of TGF-β1 was noticed as compared with both patients with stable disease and healthy control groups. Serum levels of both IL-17A and TGF-β1 have been shown to be negatively correlated with the disease duration ($r=-0.174$, $p=0.405$) and ($r=-0.057$, $p=0.788$) respectively.

Keywords: Vitiligo, IL-17A, TGF-β1.

Introduction

Vitiligo is an acquired or inherited depigmentary disorder characterized by the development of milky macules in the skin, hair and mucosal surfaces due to the selective loss of melanocytes[1].Its global prevalence has been ranging from 1-2% with no predilection for age, sex, or race, white macules may progress slowly or rapidly, some patients have reported additional depigmentation following periods of physical or emotional stress[2]. Vitiligo is classified in to three clinical types depending on the distribution and extension of lesions; localized, generalized (which is further subdivided into vulgaris and acrofacial vitiligo) and universal vitiligo[3].Several pathophysiologic theories have been suggested in order to explain the loss of epidermal melanocytes, however the autoimmune theory has been strongly implicated in the development of this disease for the following observations: association of vitiligo with autoimmune conditions, demonstration of organ-specific and circulating anti melanocyte autoantibodies in the serum of patients, infiltration and alteration in cytotoxic T cells and helper T cells in histological studies of skin biopsies[4].Naive helper T cells develop into four types, T-helper1 (Th1) which produce interferon (IFN)-γ and tumor necrosis factor (TNF)-α, Th2 which secret interleukin (IL)-4, IL-5 and IL-13,Th17 which produce IL-6 and IL-17 , and regulatory T cells (Tregs) which synthesize IL-10 and transforming growth factor β(TGF)-β[5].A novel hypothesis has been proposed that the progression of autoimmune disease, which vitiligo is one of them is partially dependent on the skewing of immune responses towards Th1 or Th17 and away from Th2 and Tregs cells, in addition the balance between Th17 and Tregs cells has been associated with the pathogenesis of several autoimmune diseases[6].

Materials and Methods

This study was carried out on 25 Iraqi vitiligo patients who were referred to AL-Kindi Teaching Hospital, and 25 apparently healthy control matched for age and gender .The mean age of patients and control subjects was 28.48±1.761 and 29.05±1.31 years respectively. Exclusion criteria includes patients on treatment with systemic steroids or immunosuppressive drugs within the last 4 weeks, as well as those with an autoimmune disease which might affect the immunological status of the patients. The level of serum cytokines (IL-17A and TGF-β1) were detected by using Enzyme Linked Immunosorbent Assay (ELISA) technique kits. Statistical Package for Social Sciences (SPSS) version 25.0 was used to analyze the data of this study which were presented in terms of mean ±Standard Error(S.E.), differences between means were assessed by one way ANOVA test and considered significant when the probability(P) value was≤ 0.05[7].

Results

Among the 25 patients in this study; females group (16 patients) shown higher incidence than males (9 patients) with a ratio equals to 1.77:1, their ages ranged from (15-45) years with a mean of (28.48±1.761) years, the duration of the disease ranged from (1-12) years with a mean of (5.28±0.59) years, no significant difference was found between patients and control groups with respect to sex and age. According to the age at onset: 12 patients (48%) had early onset vitiligo (before 30 years) while 13 patients (52%) had late onset vitiligo (after 30 years of age). The disease was active (new lesions or progression of old lesions within the three months prior to this study) in 18 patients (72%) and stable in 7 patients (28%). About 22 patients (88%) had vulgaris vitiligo while 3 patients (12%) had acrofacial vitiligo. A positive family history was found in 7 patients (28%), while the Koebner phenomenon was detected in 3 patients (12%).

Serum Level of IL-17A and TGF-β1 in patients and control groups:

Results of the current study showed that serum level of IL-17A was increased significantly (p≤0.05) while the serum level of TGF-β1 was decreased significantly (p≤0.05) in vitiligo patients as compared with healthy control (Table.1).

Table.1: Mean serum level of IL-17A and TGF-β1 (pg/ml) in the study samples.

Samples	No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Vitiligo Patients	25	114.517±7.447a	12038.16±956.22a
Healthy Control	25	69.116±4.877b	17042.39±651.59b
*Different letters=Significant difference (P≤0.05) between means.			

According to the gender of patients, it was noticed that the mean level of both IL-17A and TGF-β1 in males showed a slightly non-significant increased (p<0.05) as compared with females. Patients with early onset vitiligo had non-significant increased (p<0.05) in the mean level of both IL-17A and TGF-β1 as compared with late onset vitiligo patients, no significant differences (p<0.05) were detected in the serum levels of IL-17A and TGF-β1 in patients regarding the clinical types of generalized vitiligo (Table.2).

Table.2: Mean serum levels of IL-17A and TGF-β1 (pg/ml) according to patients' gender, age at onset and the clinical types of vitiligo.

		No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Gender	Male	9	116.568±12.993a	13235.66±1628.27a
	Female	16	113.364±9.369a	11364.56±1204.04a
Age at Onset	Early Onset	12	116.211±10.782a	123419.44±1405.07a
	Late Onset	13	112.950±10.703a	11686.20±1375.22a
Clinical Types	Vulgaris Vitiligo	22	114.981±8.357a	12038.53±1087.58a
	Acrofacial Vitiligo	3	111.114±12.758a	12035.38±1444.55a
*Similar letters=No significant difference (P> 0.05) between means.				

Patients with active disease had a significant increase ($p \leq 0.05$) in the serum level of IL-17A and a significant decrease ($p \leq 0.05$) in the serum level of TGF-β1 as compared with both patients with stable disease and healthy control groups. However no significant differences ($p < 0.05$) were recorded when patients with stable disease were compared to healthy control as regards IL-17A and TGF-β1 means serum levels (Table.3).

Table.3: Mean serum levels of IL-17A and TGF-β1 (pg/ml) according to the activity of vitiligo.

Samples	No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Patients With Active Vitiligo	18	124.833±8.565a	10574.41±1049.66a
Patients With Stable Vitiligo	7	87.991±9.747b	15802.09±1415.09b
Healthy Control	25	69.116±4.877b	17042.39±651.59b
*Similar letters=No significant difference (P>0.05) between means. *Different letters=Significant difference (P≤0.05) between means.			

According to the patients family history and Koebner phenomenon, no significant differences ($p < 0.05$) were detected in the means levels of IL-17A and TGF-β1 (Table.4).

Table.4: Mean serum levels of IL-17A and TGF-β1(pg/ml) according to patients' family history and Koebner phenomenon.

		No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Family History	Positive	7	119.816±15.221a	13573.64±2125.61a
	Negative	18	112.45±8.722a	11441.02±1063.81a
Koebner phenomenon	Present	3	111.869±3.062a	12014.52±1469.33a
	Absent	22	114.878±8.477a	12041.38±1087.14a
*Similar letters=No significant difference (P>0.05) between means.				

In patients, serum levels of both IL-17A and TGF-β1 have been shown to be negatively correlated with the disease duration (r=-0.174, p=0.405) and (r=-0.057, p =0.788) respectively (Table.5).

Table.5: Correlation between serum levels of IL-17A and TGF-β1(pg/ml) and disease duration.

		r	p	Significance
Disease Duration (years)	IL-17A(pg/ml)	-0.174	0.405	Non-significant
	TGF-β1(pg/ml)	-0.057	0.788	Non-significant

Discussion

Vitiligo is a cutaneous multifactorial disease, affects both sexes equally, higher incidence of females in this study may be result from the greater psycho-social perceived impact of the disease on their life. The presence of positive family history in first degree relative for some patients may indicate a hereditary factor for this disorder[8].The detection of T-cells infiltration in the perilesional skin of vitiligo macules in previous histopathological studies implicates the cellular immune response in the destruction of melanocytes[9].Th17 cells play a very important role in the pathogenesis of numerous autoimmune disorders by the production of proinflammatory cytokines includes:IL-17,IL-22 and IL-23, recruit neutrophils, and promote inflammation at the site of infection[10].In contrast Treg cells inhibit the immune response by the production of anti-inflammatory cytokines include IL-10 and TGF-β which suppress the activity of a variety of immune cells[11]. Several previous studies evaluate the levels of IL-17A and TGF-β1 in the serum of vitiligo patients in alternative countries and reached for the following results: Khan *et al.*[12] reported that the serum level of IL-17A in 45 Indian patients was increased significantly while the serum level of TGF-β1 was decreased significantly as compared with healthy control which came in agreement with the results of this paper. Basak *et al*.[13], on the other hand, found no significant difference in the serum level of IL-17A in 40 Turkish patients as compared with healthy control, in spite of the positive correlation between IL-17A level and extent of body area involvement. Therefore, IL-17A imbalance may suggest its involvement in the pathogenesis of vitiligo. In contrast, Zhou *et al*.[14] demonstrated an elevated level of TGF-β1 in patients with active non segmental vitiligo as compared to race-,

gender- and age-matched healthy subjects. Moreover the level of serum TGF-β1 in 38 vitiligo patients showed no significant difference in comparison to control group as established by Ghanem *et al.*[15].So TGF-β1 might play a role in the pathogenesis of vitiligo related to the suppressive function of Tregs.

Depending on the outcomes of the present study, which were similar to the findings of Elela *et al.*[16] no differences were established in the means levels of both IL-17A and TGF-β1 regarding patients' gender, age at onset, family history, Koebner phenomenon and clinical types of vitiligo, furthermore IL-17A and TGF-β1 means level were negatively correlated with disease duration, however positive correlation was observed by Tembhre *et al.*[17] in 80 Indian patients with generalized vitiligo. Patients with active vitiligo showed a significant increase in the level of IL-17A while a significant decrease in the level of TGF-β1 in comparison to patients with stable vitiligo and healthy control groups, Bhardwaj *et al.*[18] also detected a significant increase in the serum level of IL-17A in patients with active vitiligo, however TGF-β1 was also increased significantly which differs from the results of this study. Another study done by Dwivedi *et al.*[19]had been reported a significant reduction in the count and percentage of Treg cells in patients with active vitiligo as compared to those with stable vitiligo, which may explain the more reduction of serum TGF-β1 in active disease.

Interleukin-17A is a pro-inflammatory cytokine secreted by Th1 cells in the presence of IL-6/IL-21, TGF-β and IL-1β, it is implicated in the pathogenesis of many inflammatory disorders by inducing the expression of various cytokines, chemokines, adhesion molecules, and growth factors [20]. Higher expression of IL-17 mRNA in vitiliginous lesions provided an evidence for the involvement of IL-17 in the pathogenesis of vitiligo[21].It was noticed that Microphthalmia-associated transcription factor(which play a very important role in melanocytes functions and survival) was decreased in the nucleus of melanocytes when treated with 10ng/ml of IL-17A[18, 22],in addition, it was detected that melanocytes treated with 1and/or 10 ng/ml of IL-17A undergo morphological shrinking which decreased melanin production[22].IL-17A has ability to induce the production of several chemokines from keratinocytes which cause further infiltration of neutrophils, macrophages, and dendritic cells, the presence of these cells coincide with loss of melanocytes in the depigmented white macules[23].IL-17 also induces the production of vascular endothelial growth factor which facilitate the passage of melanocyte-reactive T cells to cutaneous melanocytes in the lesions and caused their destruction[24].

Transforming growth factor β1 is an important immunosuppressive cytokine produced by many cells, such as epidermal keratinocytes and Treg cells, it has the ability to inhibit the proliferation and differentiation of autoreactive CD4+ and CD8+ cells as well as B cells so it responsible for maintaining immune tolerance[25]. Regulatory T cells considered as a main source for TGF-β1 production, therefore the reduction in Tregs cells number in vitiligo patients[26] will effect on the immune tolerance to melanocyte self-antigens as well as decreased the level of TGF-β1 which came in agreement with the results of the present study. Basak *et al.*[13] suggested that the decreased level of TGF-β1 in the serum of vitiligo patients may be due to: the dominant immune response to CD8+ cells and reduction of Treg cells maturation which assisting the appearance of vitiligo lesions. According to the findings of the current study, the significant reduction in the mean serum level of TGF-β1 in patients with active disease may propose severe deficiency in TGF-β1 secretion which enhanced cell mediated immunity that cause melanocyte destruction and the appearing of white macules in patients with generalized vitiligo.

Conclusions: Increased serum level of IL-17A and decreased serum level of TGF-β1 in generalized vitiligo patients support the role of both cellular immune response and altered cytokines level in the destruction of melanocytes and the pathogenesis of vitiligo.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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