Plasma levels of interleukin-17 and transforming growth factor-beta in vitiligo patients

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ABSTRACT

Vitiligo is a common dynamic depigmentary skin disorder. Several hypotheses have been proposed to explain its underlying pathogenesis, including the autoimmune hypothesis. The objectives of the current study are to assess the plasma levels of interleukin-17 (IL-17) and transforming growth factor-beta (TGF-β) in vitiligo patients. Furthermore, we aim to estimate the relationship between the severity of vitiligo, gender and age. We assessed the levels of target cytokines in fifty vitiligo patients (VG) and twenty matched healthy controls (CG). Our data showed that the mean level of IL-17 was significantly higher in VG than in CG (P = 0.004), while TGF-β values were significantly lower in VG than in CG (P = 0.002). This modulation in the level of cytokines correlated with age and degree of disease severity but not with the sex of the patients. In conclusion the study revealed the inter-opposing action of IL-17 and TGF-β in the pathogenesis of vitiligo, as well as verified that no correlation exists between disease severity and the sex of the patient.

KEYWORDS: vitiligo, IL-17, TGF-β, autoimmune.

INTRODUCTION

Vitiligo is a common, progressive depigmentation skin disorder of unknown etiology. There is progressive loss of epidermal melanocytes. Clinically, vitiligo is manifested by appearance of well-defined, irregular-shaped pale white patches related to selective loss of cutaneous melanocytes [1, 2]. The global prevalence of vitiligo has been estimated to be 1-3% of the world’s population [3]. The disease is slightly more widespread among females. In about 50% of all patients, the disease develops before the age of twenty. The most common clinical form of vitiligo appeared in the acral areas and the face [4]. Due to its disfiguring effect vitiligo may be the source of severe psychological distress, and social stigma, especially in dark-skinned individuals and adolescent girls [5, 6].

Although the pathogenesis of vitiligo is not fully elucidated, several theories have been proposed to explain the underlying pathogenesis, including autoimmune, neurochemical, oxidative stress, and genetic theories [7].

The autoimmune theory of vitiligo proposes that a disorder in the immune system results in the destruction of melanocytes. This theory is supported by the observation that many autoimmune diseases such as autoimmune thyroid disease, psoriasis and adult type-1 diabetes are not infrequently associated with vitiligo [8, 9].

It is suggested that both components of the acquired immune system (humoral and adaptive immunity) are involved in the pathogenesis of vitiligo [10]. Concerning adaptive immunity, vitiligo is associated with a blunt increase in secretion of inflammatory cytokines and chemokines in blood and skin. This in turn stimulates the recruitment of antigen-
presenting cells (APCs) and activated T-cells, thus inducing self-antigen presentation that contributes to melanocyte destruction [3, 11-15].

Human interleukin 17 (IL-17) is a proinflammatory cytokine that belongs to the IL-17 family of cytokines. IL-17 promotes production of the chemokine CCL20, which attracts cytotoxic T cells to the peripheral tissues, which may directly kill the melanocytes [16, 17].

Transforming growth factor-beta (TGF-β) is a multipotent cytokine produced by the T-regulatory CD4+ cell subset (Treg). This potent cytokine plays an important role in maintenance of self-tolerance by suppressing proliferation of the effector T-cells [18, 19]. Dysregulation of Tregs may contribute to the pathogenesis of vitiligo through breakdown of self-tolerance to melanocyte antigens [20, 21].

The aim of this work is to assess the autoimmune hypothesis. This was done by comparing the levels of plasma cytokines in patients and matched controls. In addition we aim to estimate the relationship between gender, age and degree of vitiligo.

PATIENTS AND METHODS

Ethical approval

The study protocol was approved by the ethical committee for human experimentation of Aswan University.

Study grouping

Fifty adult patients with vitiligo and twenty sex and age-matched healthy control individuals were enrolled in this study. Vitiligo patients were randomly selected from the dermatology department at Aswan University Hospitals. Control subjects were selected from blood donors reporting to the blood transfusion centre at Aswan University.

Selection criteria of the study population

Depigmentation in the selected cases should not be the outcome of chemical agents, burns or any other traumatic causes. The patients also should be free from any associated autoimmune disease such as thyroiditis and psoriasis. Vitiligo was diagnosed by an experienced dermatologist on a clinical basis. A full medical history of the patients was taken, and a clinical examination to determine the degree of severity of vitiligo was done for every vitiligo patient.

Sample collection

Venous blood samples about 5 ml each, were collected from both groups, into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The sample was centrifuged at 3,000 rpm, and the plasma was separated within 1 hour of sample collection and then stored at -80 °C for further examinations. The stored plasma samples were allowed to thaw once at ambient temperature before analysis, to avoid repeated freezing and thawing cycles.

Enzyme-linked immunosorbent assay (ELISA)

Plasma level of IL-17 was measured using a solid-phase sandwich ELISA (R&D systems) and that of TGF-β1 (DRG Diagnostics) according to the manufacturer’s instructions. The proportional color change in the samples was measured using a Stat Fax 4200 microplate reader from GMI (Minnesota, USA).

RESULTS

Study subjects were classified into two groups; one group comprising fifty vitiligo patients (VG) of age ranging from 5 to 59 years with a mean age of 34.96 ± 10.1 and the other comprising twenty healthy individuals of age ranging from 24 to 37 years with a mean age of 31 ± 3.5, which served as the control group (CG). The percentage of males and females in both groups were set as 40% and 60% respectively.

Variability in the levels of cytokines with different severity degrees

Levels of IL-17 and TGF-β were measured in all study subjects. The mean level of IL-17 was significantly higher in VG than in CG (P ≤ 0.004). On the other hand, the mean TGF-β level was lower in VG compared to the CG (P ≤ 0.002) as shown in Table 1.

Percentage of vitiligo distribution on the whole body of each patient was assessed and then the

<table>
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<th>Parameters</th>
<th>VG (n = 50)</th>
<th>CG (n = 20)</th>
<th>P-value</th>
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<td>IL-17</td>
<td>27.36 ± 5.23</td>
<td>23.69 ± 2.21</td>
<td>≤ 0.004</td>
</tr>
<tr>
<td>TGF-β</td>
<td>13.32 ± 1.35</td>
<td>14.52 ± 1.45</td>
<td>≤ 0.002</td>
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patients were divided into five different groups representing percentage of body affected, based on the affected area and width of distribution, as: 5%, 9%, 18%, 36% and 50%. Measurements of cytokines in different cases showed a gradual elevation of IL-17 levels with increase in severity while TGF-β showed reduced levels with increase in severity, in the VG as shown in Table 2.

### Correlation of cytokines with age and sex

Correlation of age and sex with cytokine elevation, at different degrees of severity in the VG was calculated. Levels of IL-17 showed a moderate direct correlation with age ($r = 0.433$), while a moderate reverse correlation between TGF-β and age was observed ($r = -0.335$). On the other hand, there was no correlation between IL-17 or TGF-β and sex ($P = 0.013; P = 0.308$, respectively) as shown in Table 3.

### DISCUSSION

The current study included fifty patients with vitiligo who were treated in Aswan University hospital and 20 healthy individuals as controls. Levels of IL-17 in patients were not different from that of control in Sudanese and Iranian patients [22, 23]. On the other hand, other studies have reported elevation of IL-17 levels in Egyptian patients with vitiligo [24, 25]. From these studies, it is obvious that modulation of IL-17 levels in patients with vitiligo is controversial.

The present study is in consensus with an Indian study which showed a significant elevation of IL-17 in the VG compared to the control subjects [24-26]. In addition, we found a high degree of correlation between size of diseased skin and level of IL-17, as previously reported [24, 27]. Furthermore, our study reported that TGF-β was significantly lower in VG patients than in CG, which indicates a dysregulation of Tregs, and hence supports the autoimmune hypothesis of the disease. These data are in agreement with other studies which indicated a disturbance of immune homeostasis when the level of TGF-β was downregulated [27, 28].

Our data also indicated a reverse correlation between the levels of TGF-β and degree of severity in vitiligo patients, which is contradictory to other studies that found no significant difference in the levels of TGF-β, between the lesional and non-lesional skin of patients [29]. Moreover, the data in the current study is in consensus with that of a previous study in a Korean cohort showing a strong reverse correlation between TGF-β and the degree of vitiligo [28].

Our study also revealed that there was no relation between TGF-β levels and the sex of patients. This in agreement with the results of other studies which showed no relation between TGF-β levels and the sex of patients [29]. On the other hand, our data recorded a correlation between TGF-β and age of patients.

In this study the opposing values of IL-17 and TGF-β levels in the plasma of vitiligo patients revealed a moderate reverse correlation between

<table>
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<tr>
<td></td>
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<td>IL-17</td>
<td>25.19 ± 2.90</td>
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<tr>
<td>TGF-β</td>
<td>14.52 ± 1.1</td>
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<td>IL-17</td>
<td>0.416</td>
<td>0.220</td>
<td>476 ≤ 0.0001</td>
</tr>
<tr>
<td>TGF-β</td>
<td>0.004</td>
<td>-0.068</td>
<td>-725 ≤ 0.0001</td>
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these two cytokines. This reverse correlation coincides with the expected roles of these cytokines in the pathogenesis of vitiligo, supporting the autoimmune hypothesis of this disease. In contrast with our finding, a study carried out by Zhou and colleagues in the USA reported a direct relationship between the levels of IL-17 and TGF-β1 in sera of vitiligo patients [30].

CONCLUSION

In conclusion, according to our study there were significant differences in the levels of IL-17 and TGF-β between VG and CG and a reverse correlation between the expression of these two cytokines. These findings suggest the important roles of these two cytokines in the pathogenesis of vitiligo, which supports the autoimmune hypothesis of diseases.

CONSENT

The participants were informed about the purpose of the research and gave written informed consent to participate.

FUNDING

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES