Interleukin-6: a possible inflammatory link between vitiligo and type 1 diabetes

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ABSTRACT

Vitiligo is a pigmentation disorder of unknown aetiology, but it has been reported in association with other autoimmune diseases including type 1 diabetes mellitus (T1D). Vitiligo and T1D share a common theory of autoimmunity, but still an inflammatory link between them remains to be investigated. This study investigates the status and contribution of the inflammatory markers tumour necrosis factor-α (TNFα), interleukin (IL)-6 and IL-1 in patients with vitiligo, T1D and vitiligo-associated T1D (Vt-T1D). The data showed that sera from Vt-T1D patients (n=21) had higher levels of TNFα, IL-6 and IL-1β compared with vitiligo patients (n=39), T1D patients (n=37) or controls (n=42). Interestingly, serum levels of IL-6 were found to be significantly higher in Vt-T1D patients compared with the levels of TNFα and IL-1β. These data also showed that IL-6 was high in Vt patients as compared to the levels of TNFα and IL-1β, whereas in T1D patients, IL-6 and TNFα were almost the same but were higher than IL-1β. In conclusion, this is the first study to show an inflammatory link between vitiligo and T1D. The data conclude that IL-6 plays an important role in the pathogenesis of Vt-T1D patients and is likely to gain favour as a therapeutic target in these patients.

KEY WORDS: Cytokines.
Diabetes mellitus, type 1.
Inflammation.
Interleukin-6.
Vitiligo.

Introduction

Vitiligo is a common dermatological disorder, characterised by the appearance of white macules on the skin, mucosa or hair,[1] The pathobiology of vitiligo has been disputed and has been a magnet for endless speculation, with different schools of thought ranging from the concept that vitiligo essentially is a free-radical disorder to that of vitiligo being a primary autoimmune disease.[2]

Type 1 diabetes mellitus (T1D) is a well-established autoimmune disease characterised by the targeted destruction of the insulin-secreting β-cells within the pancreatic islet.[3] The aetiology of vitiligo and T1D remains obscure; however, there is a general consensus that vitiligo and T1D are T-cell-mediated disorders that result from immune dysfunction, with subsequent loss of melanocytes in vitiligo.[3,4] In T1D the patient's immune dysfunction causes subsequent loss of tolerance to β-cell antigens and destructive lymphocytic infiltration of the islets. This, in turn, leads to hyperglycaemia.[5]

The clinical association between vitiligo and various organ-specific autoimmune diseases including T1D strongly suggests that vitiligo also has an autoimmune aetiology.[6,7] Similarly, the increased prevalence of autoantibodies in subjects with vitiligo supports the hypothesis of an autoimmune aetiology.[8,9] The occurrence of vitiligo in T1D may be as a result of a basic autoimmune disturbance in the same patient affecting two organ systems, or an injury to melanocytes may be caused, resulting in the release of an antigenic substance, anti-melanocyte antibody formation, inhibition of melanogenesis and occurrence of vitiligo.[10]

It is well established that vitiligo and T1D are associated with a common autoimmune aetiology.[3,5,7,8] Autoimmunity and also familial hereditary tendencies occur in both diseases, suggesting that both diseases are genetic linked.[10,11] In addition, vitiligo and T1D are also associated with neuropathic complications and numerous multiple pathogenetic mechanisms including vascular and non-vascular pathogenetic actions, the products of oxidative stress, free radical generation and release of various growth factors.[12–14]

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Inflammation is an essential physiological response to diverse pathological events, such as pathogen invasion, tissue injury and other irritants. The successful resolution of such insults requires rapid infiltration of cells of the innate and adaptive immune system to the site of inflammation, and their subsequent activation.[15] The infiltrating cells, in dialogue with the affected tissue, produce various inflammatory mediators (e.g., cytokines) that regulate the multicellular inflammatory immune response in a highly coordinated manner.

This inflammatory process depends on massive changes in gene expression and protein secretion.[15,16] As a consequence, invading pathogens and defective cells are eliminated in a concerted action, thereby limiting tissue damage and enabling tissue regeneration. However, the requirement for a fast and strong reaction on threats makes the inflammatory response sensitive to aberrant activation by additional cellular stress events that can result in an increase in tissue damage or even cause chronic inflammation or autoimmunity.[17]

Our understanding of the pathogenesis of vitiligo and

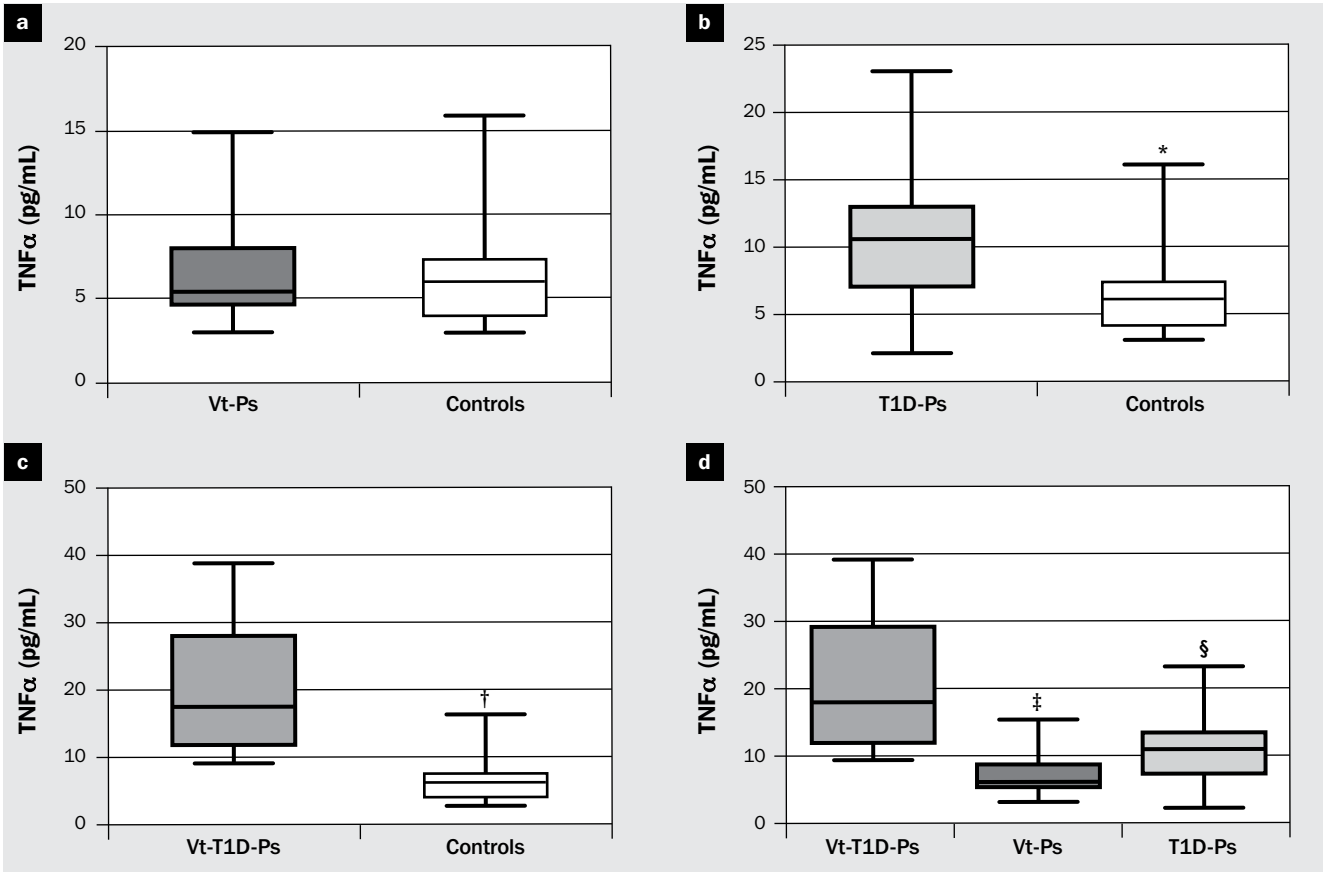

Fig. 1. Levels of tumour necrosis factor-α in vitiligo, type 1 diabetes and vitiligo associated type 1 diabetes patients. **a)** TNFα levels in serum samples of patients with vitiligo (Vt-Ps) and healthy controls. Vt-Ps versus controls, $P>0.05$. **b)** TNFα levels in serum samples of patients with type 1 diabetes (T1D-Ps) and controls, $^{*}P<0.01$ versus T1D-Ps. **c)** TNFα levels in serum samples of vitiligo patients associated with type 1 diabetes (Vt-T1D-Ps) and controls, $^{\dagger}P<0.01$ versus Vt-T1D-Ps. **d)** TNFα levels in serum samples of the Vt-T1D-Ps, Vt-Ps and T1D-Ps, $^{\ddagger}P<0.0001$ versus Vt-T1D-Ps, $^{\S}P<0.05$ versus Vt-T1D-Ps.

T1D has profited from the analysis of altered CD4-positive T-cell function in both diseases,[18,19] but the major cytokines produced by this populations of cells have not been completely studied in vitiligo as well as in T1D. Previous studies of serum cytokine concentrations in vitiligo and T1D are few and results are contradictory.[20,21] Furthermore, no information is available to show the inflammatory link in these patients.

The present study aims to investigate the role of Th1 cells in producing the most potent pro-inflammatory cytokines in vitiligo patients associated with T1D. Sera are assayed for TNFα, IL-1β and IL-6 in these patients and the levels of these biomarkers cytokines are compared in patients with vitiligo or T1D in order to find out whether or not these inflammatory mediators play a role in the pathogenesis of these disorders.

Materials and methods

The present study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for human samples and approved by the local ethical committee. Study subjects were recruited through the dermatology and diabetes out-patient clinics of Qassim University, Buraidah, KSA, and informed consent was obtained from each subject.

The study group included 39 patients with non-segmental vitiligo (14 female, 25 male; age range: 9–58 years [mean: 29.2±18.8 years]). The study group included 37 patients with type 1 diabetes mellitus (19 female, 18 male; age range: 20–45 years [mean: 33.1±16.2 years]). The study group also included 21 patients with vitiligo and a history of type 1 diabetes (nine female, 12 male; age range: 13–23 years [mean: 26.2±16.9 years]).

The control group comprised 42 healthy subjects (27 male, 15 female; age range: 18–48 years [mean: 43±17 years]). The racial/ethnic and gender compositions of the patient groups were comparable with those of the control group. Venous blood samples from the control subjects and vitiligo patients were collected and stored in small aliquots at –80°C until analysed.

Over-production of TNFα, IL-6 or IL-1β in the serum samples of studied groups were quantified by specific sandwich enzyme-linked immunosorbent assay (ELISA), performed using the serum samples of studied groups according to the instructions of the manufacturer (GenWay Biotech, San Diego, USA). Plates were read at 450 nm using an automatic microplate reader (Anthos Zenyth 3100 Multimode Detectors, Salzburg, Austria).

Statistical analysis

All measurements were performed in duplicate or triplicate using test and control sera. Statistical comparisons were

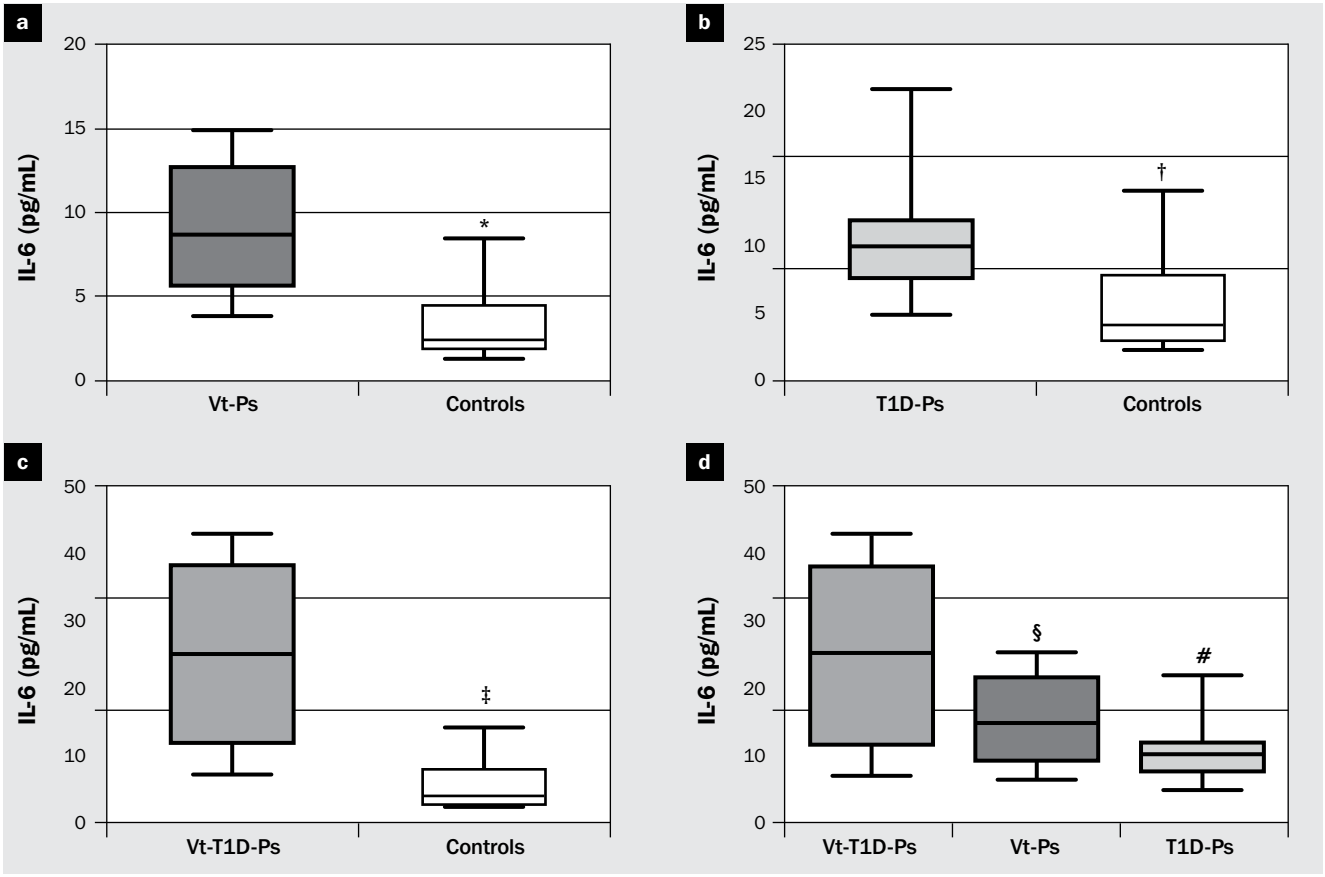

Fig. 2. Interleukin-6 in vitiligo, type 1 diabetes and vitiligo associated type 1 diabetes patients. **a)** IL-6 levels in serum samples of patients with vitiligo (Vt-Ps) and healthy controls, $^{*}P<0.001$ versus Vt-Ps. **b)** IL-6 levels in serum samples of patients with type 1 diabetes (T1D-Ps) and controls, $^{\dagger}P<0.05$ versus T1D-Ps. **c)** IL-6 levels in serum samples of vitiligo patients associated with type 1 diabetes (Vt-T1D-Ps) and controls, $^{\ddagger}P<0.0001$ versus Vt-T1D-Ps. **d)** IL-6 levels in serum samples of Vt-T1D-Ps, Vt-Ps and T1D-Ps, $^{\S}P<0.01$ versus Vt-T1D-Ps, $^{\#}P<0.001$ versus Vt-T1D-Ps.

performed using Graph Pad Prism-5 (San Diego, USA) and one paired two-tailed *t*-test with one-way ANOVA analysis followed by Tukey's/Mann-Whitney post-hoc analysis or two-way ANOVA followed by Bonferroni post-hoc tests. $P<0.05$ was considered significant. Values shown are mean±SEM unless stated otherwise.

Results

TNFα in patients with vitiligo and history of type 1 diabetes

Tumour necrosis factor-α is a major pro-inflammatory cytokine and generally serves as a biomarker of inflammation.[22] In an attempt to understand the role of inflammation in the pathogenesis of vitiligo patients associated with type 1 diabetes mellitus, serum levels of TNFα were measured in patients with vitiligo (Vt), type 1 diabetes (T1D) and in those vitiligo patients who were associated with T1D (Vt-T1D), and levels were compared with their respective healthy controls. As shown in Figure 1a, the serum levels of TNFα were slightly higher in Vt patients in comparison with healthy controls ($P>0.05$). The average (±SD) TNFα levels in Vt patients and controls were 6.5±3.25 pg/mL and 6.43± 3.50 pg/mL, respectively. Serum TNFα was significantly increased in T1D patients ($P<0.05$) compared with controls. The average (±SD) serum TNFα in T1D patients and healthy controls were 11.1±5.40 pg/mL and 6.43±3.50 pg/mL, respectively (Fig. 1b). The average (±SD) serum TNFα in Vt-T1D patients and controls were 19.3±8.93 pg/mL and 6.43±3.50 pg/mL, respectively (Fig. 1c). Interestingly, the increases were significantly greater in patients with Vt-T1D compared with Vt or T1D patients (Fig. 1d). The raised serum TNF in Vt-T1D patient suggests that inflammation is increased in these patients and points to an inflammatory link between vitiligo and T1D.

Interleukin-6 in patients with vitiligo associated with type 1 diabetes

Interleukin-6 is another important pro-inflammatory mediator and a most effective therapeutic target in various autoimmune diseases;[23] therefore, for confirmation of an inflammatory link between vitiligo and type 1 diabetes, serum levels of IL-6 were determined. Serum IL-6 was found to be significantly higher in Vt patients compared with healthy controls ($P<0.001$). The average (±SD) IL-6 levels in Vt patients and controls were 18.2±7.33 pg/mL and 6.64±4.25 pg/mL, respectively (Fig. 2a). Serum IL-6 was also higher in T1D patients compared with controls. The average (±SD) serum IL-6 levels in T1D patients and healthy controls were 12.4±5.08 pg/mL and 6.64±4.25 pg/mL, respectively (Fig. 2b). Levels were also significantly higher in Vt-T1D patients compared to controls. The average (±SD) serum IL-6 levels in Vt-T1D patients and healthy controls were 29.4±15.24 pg/mL and 6.64±4.25 pg/mL, respectively

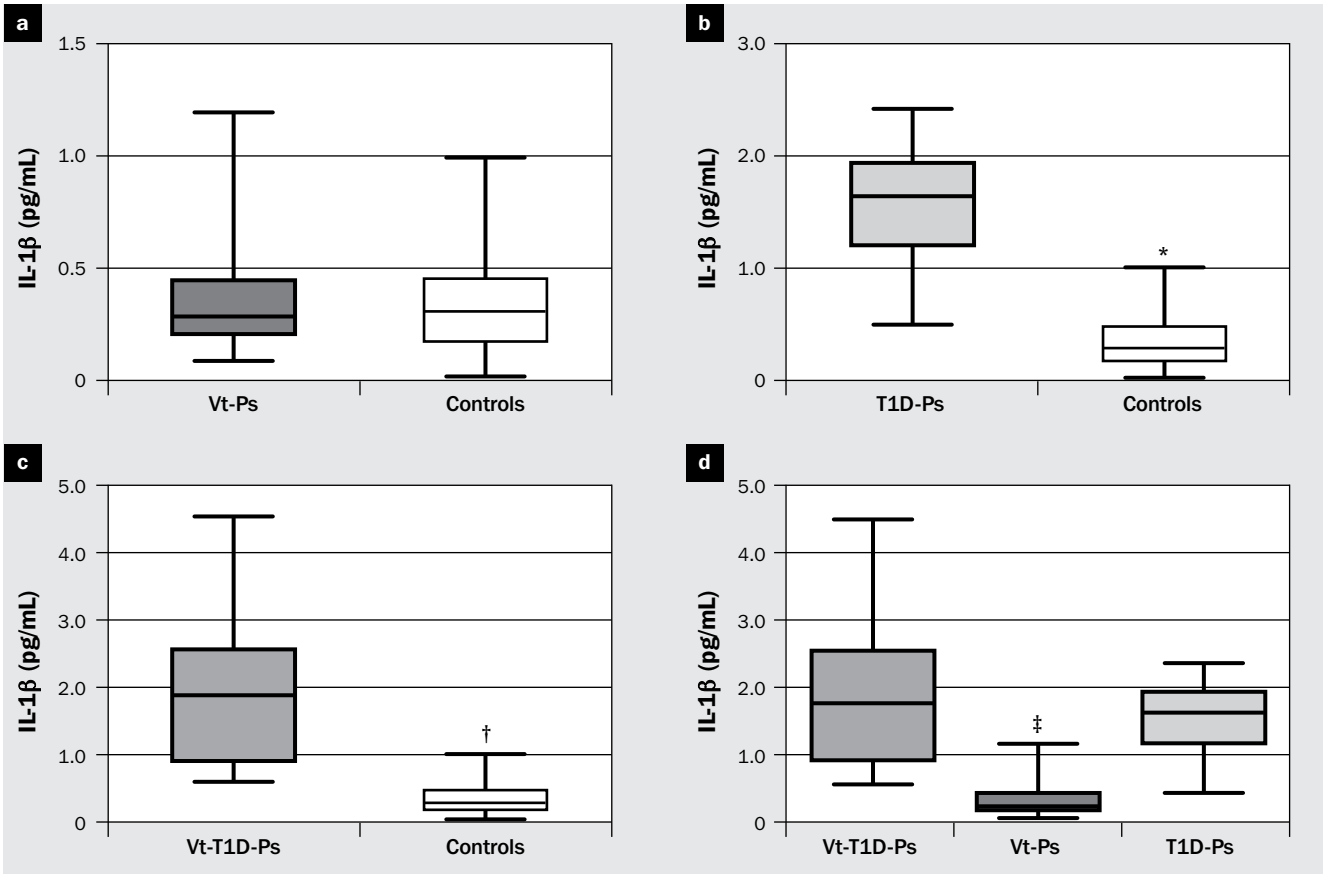

Fig. 3. Interleukin-1 in vitiligo, type 1 diabetes and vitiligo associated type 1 diabetes patients. **a)** IL-1β levels in serum samples of patients with vitiligo (Vt-Ps) and healthy controls, Vt-Ps versus controls $P>0.05$. **b)** IL-1β levels in serum samples of patients with type 1 diabetes (T1D-Ps) and controls, $^{*}P<0.05$ versus T1D-Ps. **c)** IL-1β levels in serum samples of vitiligo patients associated with type 1 diabetes (Vt-T1D-Ps) and controls, $^{\dagger}P<0.001$ versus Vt-T1D-Ps. **d)** IL-1β levels in serum samples of Vt-T1D-Ps, Vt-Ps and T1D-Ps, $^{\ddagger}P<0.001$ versus Vt-T1D-Ps, Vt-T1D-Ps versus T1D-Ps $P<0.01$.

(Fig. 2c). Interestingly, IL-6 levels were also significantly higher in patients with Vt-T1D compared with Vt or T1D patients (Fig. 2d). The raised serum levels of IL-6 in Vt-T1D patients suggests that inflammation is increased in these patients and indicates an inflammatory link between vitiligo and T1D.

Interleukin-1 in vitiligo patients with history of type 1 diabetes

Interleukin-1β is another major pro-inflammatory cytokine that serves as a biomarker of inflammation in numerous diseases.[24] Therefore, serum IL-1β levels were determined in vitiligo patients associated with type 1 diabetes mellitus. Levels in Vt patients were found to be similar to healthy controls ($P>0.05$). The average (±SD) IL-1β level in Vt patients and controls were 0.37±0.29 pg/mL and 0.36±0.27 pg/mL, respectively (Fig. 3a). Serum IL-1β was significantly increased in T1D patients ($P<0.05$) compared with controls. The average (±SD) serum IL-1β in T1D patients and healthy controls were 1.58±0.52 pg/mL and 0.36±0.27 pg/ml, respectively (Fig. 3b). IL-1β levels were also found to be significantly higher in Vt-T1D compared to healthy controls (Fig. 3c). IL-1β levels were found to be significantly higher in patients with Vt-T1D compared to Vt

Table 1. Levels of inflammatory cytokines (pg/mL) in sera from patients with vitiligo, type 1 diabetes and vitiligo patients associated with type 1 diabetes.

Cytokine	Vt patients ($n=39$)	T1D patients ($n=37$)	Vt-T1D patients ($n=21$)	Controls ($n=42$)
TNFα	6.5±3.25	11.1±5.40	19.3±8.93	6.43±3.50
IL-6	18.2±7.33	12.4±5.08	29.4±15.24	6.64±4.25
IL-1β	0.37±0.29	1.58±0.52	1.93±1.17	0.36±0.27

Vt: vitiligo; T1D: type 1 diabetes; Vt-T1D: vitiligo patients associated with type 1 diabetes.

TNF-α: Vt versus control $P>0.05$; T1D versus control $P<0.01$; Vt-T1D versus control $P<0.001$; Vt-T1D versus Vt or T1D $P<0.05$.

IL-6: Vt versus control $P<0.001$; T1D versus control $P<0.05$; Vt-T1D versus control $P<0.0001$; Vt-T1D versus Vt or T1D $P<0.01$.

IL-1β: Vt versus control $P>0.05$; T1D versus control $P<0.05$; Vt-T1D versus control $P<0.001$; Vt-T1D versus Vt or T1D $P<0.05$.

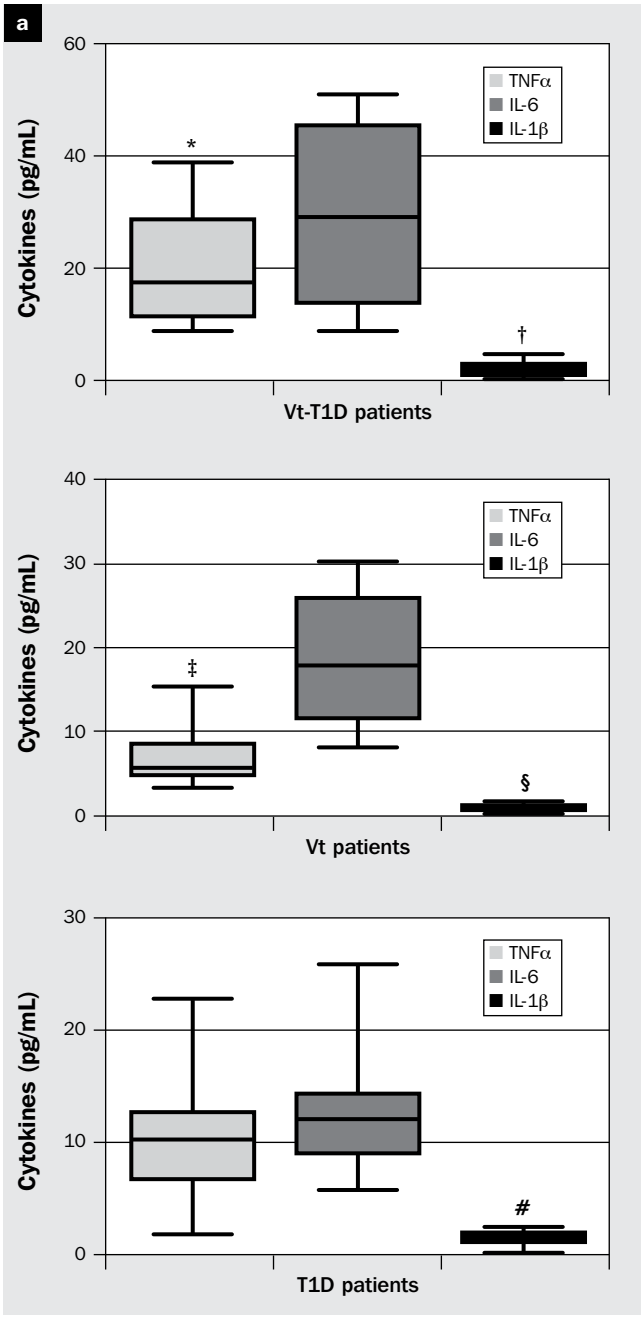

Fig. 4. Levels of TNFα, interleukin-6 and interleukin-1β in **a)** vitiligo patients with a history of type 1 diabetes (Vt-T1D-Ps), **b)** vitiligo patients (Vt-Ps) and **c)** type 1 diabetes patients (T1D-Ps). In Vt-T1D patients, $^{*}P<0.01$ versus IL-6, $^{\dagger}P<0.0001$ versus IL-6. In Vt patients, $^{\ddagger}P<0.001$ versus IL-6, $^{\S}P<0.0001$ versus IL-6. In T1D patients, $^{\#}P<0.001$ versus IL-6.

or T1D patients. The average (±SD) IL-1β levels in patients with Vt-T1D, Vt and T1D were 1.93±1.17 pg/mL, 0.37±0.29 pg/mL and 1.58±0.52 pg/mL, respectively (Fig. 3d).

TNFα, IL-6 and IL-1β in patients with vitiligo associated with type 1 diabetes

As shown in Figure 4a, serum IL-6 level was found to be significantly higher in Vt-T1D patients compared with levels of TNFα ($P<0.01$) and IL-1β ($P<0.0001$). These data also show that IL-6 was higher in Vt patients compared to the levels of TNFα and IL-1β (Fig. 4b). In T1D patients, serum levels of IL-6 and TNFα were similar, but levels of IL-1β were significantly lower compared to levels of IL-6 and TNFα (Fig. 4c). Serum levels of TNFα, IL-1β and IL-6 in Vt, T1D and Vt-T1D patients are summarised in Table 1.

Discussion

The authors believe this study to be the first that demonstrates an inflammatory link between vitiligo and type 1 diabetes mellitus. Vitiligo is known as an idiopathic acquired depigmentation disorder characterised by the loss of functional melanocytes from the epidermis, and is reported to develop in T1D patients.[25–27] The incidence of vitiligo in T1D is 1–7% in literature and vitiligo may precede the onset of T1D.[28]

Dawber[26] suggested that T1D should be excluded in all cases of late-onset vitiligo. Among 457 diabetic subjects attending an out-patient clinic, 9% had vitiligo.[29] In another series, 10% of 100 patients withT1D had vitiligo compared with and incidence of 1% in the general population.[30] Vitiligo is a consistent finding in literature concerning skin complications of diabetes. Recent observations support the role of altered cellular immunity, autoimmunity and a role for cytokines in the pathogenesis of vitiligo and T1D.[4–7] While it is established that both diseases share a common theory of autoimmunity,[3,8] an inflammatory link between these conditions has not been addressed previously.

Cytokines are regulators of host responses to infection, immune responses, inflammation and trauma. Pro-inflammatory cytokines act to exacerbate disease, whereas anti-inflammatory cytokines serve to reduce inflammation and promote healing.[31] Attention has focused on blocking of pro-inflammatory cytokines, which are harmful to the host, particularly during overwhelming infection.[17,32] TNFα, IL-1β and IL-6 are pro-inflammatory cytokines that produce fever, inflammation, tissue destruction and, in some cases, shock and death.[22–24] Reducing the biological activity of TNFα, IL-1β and IL-6 is accomplished by several different, highly-specific strategies that involve neutralising antibodies, soluble receptors, receptor antagonist, and inhibitors of proteases that convert inactive precursors to active, mature molecules.[33]

Blocking TNFα, IL-1β or IL-6 has been highly successful various autoimmune and chronic inflammatory disorders.[34] There is growing evidence that cytokines are important in the onset of autoimmunity and may play a role in skin depigmentation,[35] and also play a role in the destruction of insulin-producing β-cells.[36] The investigation by Swope *et al*.[37] revealed that inflammatory cytokines (TNFα, IL-1β and IL-6) inhibited melanocyte proliferation. However studies concerning cytokine expression and production in patients with vitiligo have been limited and the results contradictory.[35,38] In T1D patients, cells mediating innate as well as adaptive immunity infiltrate pancreatic islets, thereby generating an aberrant inflammatory process termed insulitis characterised by pathological autoantibody production and autoreactive T cells.[39] In cooperation with infiltrating innate immune cells, which secrete high levels of pro-inflammatory cytokines (IL-1β and TNFα), effectors T-cells trigger the destruction process of β-cells.[36]

All these studies clearly indicate that inflammation plays an important role in the pathogenesis of both vitiligo and T1D. Despite the power of modern inflammatory approaches

and persistent investigative efforts, an inflammatory link between vitiligo and T1D remains an enigma and the inflammatory mediator (or mediators) triggering these autoimmune disorders remain to be identified.

In the present study, serum levels of TNFα, IL-1β and IL-6 were measured in patients with vitiligo, type 1 diabetes and vitiligo-associated T1D. The results showed a significant increase in the production of IL-6, but diminished TNFα and IL-1β release in patients with vitiligo. These results are fully supported by previous studies.[38] All together, the data suggest that IL-6 plays an important role in melanocytic cytotoxicity. Moretti *et al*.[40] tested IL-6 and TNFα, which inhibit melanocyte activity, and found increased levels of IL-6 and TNFα in the epidermis of lesional skin. Another study revealed increased levels of serum IL-6 in vitiligo, supporting the hypothesis that IL-6 may play a role in the immunological events in the pathogenesis of vitiligo, although alterations in the serum levels of IL-1β, and TNFα could not be found.[41] However, in T1D patients there is little and somewhat conflicting information available on serum IL-6 in the diabetic state.[42]

Serum levels of IL-6 have been found to be normal[43] or higher in T1D patients compared with those in control subjects.[44] Moreover, some studies suggest that IL-6 participates in the initiation and acceleration of the chronic inflammation process and could contribute to development of secondary complications in T1D patients.[45,46] The results presented here are in full agreement with this view as significantly higher levels of IL-6 were found in Vt-T1D patients compared with controls. However, the study also found higher levels of IL-6 in T1D patients not associated with vitiligo compared to controls, but the levels were significantly low when compared with Vt-T1D patients. Thus, IL-6 may be a link between vitiligo and T1D patients.

Recent reports suggest that the pancreas participates in TNFα production, and that the islets are predominantly responsible for such synthesis. IL-1β and TNFα are important cytokines in β-cell lysis in T1D, while IL-1 receptor antagonist (IL-1ra) is considered protective by blocking the effects of IL-1β.[47] *In vitro*, TNFα and IL-1β inhibit insulin release from islet β-cells. It appears that the process of autoimmune aggression against β-cells, and its effect on insulin release and glucose homeostasis, is a slow and chronic process. However, the production of these cytokines, and consequently the degree of β-cell destruction, in a genetically susceptible subject might be enhanced by several factors including viral infection.[48]

The pro-inflammatory cytokines IL-1α and TNFα may play important roles alone or in combination in the pathogenesis of T1D.[49] IL-1β and TNFα levels can be used as indicators of continuing autoimmune aggression against β-cells before the development of extensive β-cell destruction.[48,50] To date, results regarding levels and/or production of TNFα and IL-1β have been inconclusive and are reported to be increased,[51] decreased[52] or unchanged.[48,53] The effects of glycaemic control on cytokines are consistent in several studies.[51,54] In the present study, circulating TNFα, and IL-1β were determined as markers of the inflammatory response, and data showed that serum levels of TNFα and IL-β were higher in T1D patients compared to controls. These results support to the view that these cytokines play a role in autoimmune destruction of β-cells.

To validate the central hypothesis that inflammation is an important link between vitiligo and T1D, the most potent inflammatory cytokines (TNFα, IL-1β and IL-6) were estimated in serum samples of patients with Vt-T1D, and results were compared with vitiligo or T1D patients. The data showed that all tested cytokines were higher in the Vt-T1D patients compared to vitiligo or T1D patients groups, but serum levels of IL-6 were much higher in Vt-T1D patients when compared with the levels of TNFα and IL-1β. This clearly indicates that IL-6 not only plays a role in the pathogenesis of Vt-T1D patients but also shows a positive association with vitiligo and type 1 diabetes.

In conclusions, this is the first study to show an inflammatory link between vitiligo and type 1 diabetes, with data demonstrating the role of TNFα, IL-6 and IL-1β in vitiligo-associated type 1 diabetes. Furthermore, IL-6 plays an important inflammatory role in the pathogenesis of Vt-T1D patients, but it is not clear to what extent IL-6 mediates the pro-inflammatory activity in vitiligo or type 1 diabetes pathogenesis and its utility as a monotherapy in these patients. The results presented here support the need for further studies. □

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References

1 Nicolaidou E, Katsambas AD. Pigmentation disorders: hyperpigmentation and hypopigmentation. *Clin Dermatol* 2014; **32** (1): 66–72.

2 Laddha NC, Dwivedi M, Mansuri MS *et al*. Vitiligo: interplay between oxidative stress and immune system. *Exp Dermatol* 2013; **22** (4): 245–50.

3 Donath MY, Hess C, Palmer E. What is the role of autoimmunity in type 1 diabetes? A clinical perspective. *Diabetologia* 2014; **57** (4): 653–5.

4 Malhotra N, Dytoc M. The pathogenesis of vitiligo. *J Cutan Med Surg* 2013; **17** (3): 153–72.

5 Boitard C. Pancreatic islet autoimmunity. *Presse Med* 2012; **41** (12 Pt 2): e636–50.

6 Witek PR, Witek J, Pa kowska E. Type 1 diabetes-associated autoimmune diseases: screening, diagnostic principles and management (in Polish). *Med Wieku Rozwoj* 2012; **16** (1): 23–34.

7 Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res* 2003; **16** (2): 90–100.

8 Kasumagic-Halilovic E, Ovcina-Kurtovic N, Jukic T, Karamehic J, Begovic B, Samardzic S. Vitiligo and autoimmunity. *Med Arch* 2013; **67** (2): 91–3.

9 Kemp EH, Gavalas NG, Gawkrodger DJ, Weetman AP. Autoantibody responses to melanocytes in the depigmenting skin disease vitiligo. *Autoimmun Rev* 2007; **6** (3): 138–42.

10 Olasode OA. Why vitiligo in diabetes? *EDOJ* 2005; **1** (2): 8.

11 Harsoulis P, Kanakoudu-Tsakalidis F, Vyzantiadis A, Minas A, Cassimos C. Autoimmunity and vitiligo. *Arch Dermatol* 1978; **114** (10): 1554.

12 Bose SK. Probable mechanisms of loss of Merkel cells in completely depigmented skin of stable vitiligo. *J Dermatol* 1994; **21** (10): 725–8.

13 Singh R, Kishore L, Kaur N. Diabetic peripheral neuropathy: current perspective and future directions. *Pharmacol Res* 2014; **80**: 21–35.

14 Deo SS, Bhagat AR, Shah RN. Study of oxidative stress in peripheral blood of Indian vitiligo patients. *Indian Dermatol Online J* 2013; **4** (4): 279–82.
15 Matsushima K, Terashima Y, Toda E, Shand F, Ueha S. Chemokines in inflammatory and immune diseases. *Inflamm Regen* 2011; **31** (1): 11–22.
16 Ceciliani F,V Giordano A, Spagnolo V. The systemic reaction during inflammation: the acute-phase proteins. *Protein Pept Lett* 2002; **9** (3): 211–23.
17 Rasheed Z, Haqqi TM. Update on targets of biologic therapies for rheumatoid arthritis. *Curr Rheumatol Rev* 2008; **4** (4): 246.
18 Moftah NH, El-Barbary RA, Ismail MA, Ali NA. Effect of narrow band-ultraviolet B on CD4(+) CD25high FoxP3(+) T-lymphocytes in the peripheral blood of vitiligo patients. *Photodermatol Photoimmunol Photomed* 2014; **30** (5): 254–61.
19 Sarikonda G, Pettus J, Phatak S *et al*. CD8 T-cell reactivity to islet antigens is unique to type 1 while CD4 T-cell reactivity exists in both type 1 and type 2 diabetes. *J Autoimmun* 2014; **50**: 77–82.
20 Taïeb A. Vitiligo as an inflammatory skin disorder: a therapeutic perspective. *Pigment Cell Melanoma Res* 2012; **25** (1): 9–13.
21 Nepom GT, Ehlers M, Mandrup-Poulsen T. Anti-cytokine therapies in T1D: concepts and strategies. *Clin Immunol* 2013; **149** (3): 279–85.
22 Bradley JR. TNF-mediated inflammatory disease. *J Pathol* 2008; **214** (2): 149–60.
23 Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006; **8** (Suppl 2): S3.
24 Ren K, Torres R. Role of interleukin-1beta during pain and inflammation. *Brain Res Rev* 2009; **60** (1): 57–64.
25 Chhabra N, Pandhi D, Verma P, Singal A. Pleomorphic cutaneous sarcoidosis confined to lesions of vitiligo vulgaris in a patient with type 1 diabetes mellitus. *Indian J Dermatol Venereol Leprol* 2012; **78** (6): 754–6.
26 Dawber RP. Vitiligo in mature-onset diabetes mellitus. *Br J Dermatol* 1968; **80** (5): 275–8.
27 Gould IM, Gray RS, Urbaniak SJ, Elton RA, Duncan LJ. Vitiligo in diabetes mellitus. *Br J Dermatol* 1985; **113** (2): 153–5.
28 Lerner AB. Vitiligo *J Invest Dermatol* 1959: **32** (2, Part 2): 285–310.
29 Romano G, Moretti G, Benedetto A *et al*. Skin lesions in diabetes mellitus: prevalence and clinical correlations. *Diabetes Res Clin Pract* 1998; **39** (2): 101–6.
30 Wahid Z, Kanjee A. Cutaneous manifestation of diabetes mellitus. *J Pak Med Assoc* 1998; **10**: 304–5.
31 Dinarello CA. Historical insights into cytokines. *Eur J Immunol* 2007; **37** (Suppl 1): S34–45.
32 Alghasham A, Rasheed Z. Therapeutic targets for rheumatoid arthritis: progress and promises. *Autoimmunity* 2014; **47** (2): 77–94.
33 Vilcek J, Feldmann M. Historical review: Cytokines as therapeutics and targets of therapeutics. *Trends Pharmacol Sci* 2004; **25** (4): 201–9.
34 Tayal V, Kalra BS. Cytokines and anti-cytokines as therapeutics–an update. *Eur J Pharmacol* 2008; **579** (1–3): 1–12.
35 Moretti S, Spallanzani A, Amato L *et al*. New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. *Pigment Cell Res* 2002; **15** (2): 87–92.
36 Baumann B, Salem HH, Boehm BO. Anti-inflammatory therapy in type 1 diabetes. *Curr Diab Rep* 2012; **12** (5): 499–509.
37 Swope VB, Abdel-Malek Z, Kassem LM, Nordlund JJ. Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melanocyte proliferation and melanogenesis. *J Invest Dermatol* 1991; **96** (2): 180–5.
38 Yu HS, Chang KL, Yu CL *et al*. Alterations in IL-6, IL-8, GM-CSF, TNF-alpha, and IFN-gamma release by peripheral mononuclear cells in patients with active vitiligo. *J Invest Dermatol* 1997; **108** (4): 527–9.
39 Bailey-Bucktrout SL, Martinez-Llordella M, Zhou X *et al*. Self-antigen-driven activation induces instability of regulatory T cells during an inflammatory autoimmune response. *Immunity* 2013; **39** (5): 949–62.
40 Morelli JG, Norris DA. Influence of inflammatory mediators and cytokines on human melanocyte function. *J Invest Dermatol* 1993; **100** (2 Suppl): 191S–195S.
41 Tu CX, Gu JS, Lin XR. Increased interleukin-6 and granulocyte-macrophage colony stimulating factor levels in the sera of patients with non-segmental vitiligo. *J Dermatol Sci* 2003; **31** (1): 73–8.
42 Wegner M, Araszkiewicz A, Piorunska-Stolzmann M, Wierusz-Wysocka B, Zozulinska-Ziolkiewicz D. Association between IL-6 concentration and diabetes-related variables in DM1 patients with and without microvascular complications. *Inflammation* 2013; **36** (3): 723–8.
43 Kulseng B, Vatten L, Espevik T. Soluble tumor necrosis factor receptors in sera from patients with insulin dependent diabetes mellitus: relations to duration and complications of disease. *Acta Diabetol* 1999; **36** (1–2): 99–105.
44 Targher G, Zenari L, Bertolini L, Muggeo M, Zoppini G. Elevated levels of interleukin-6 in young adults with type 1 diabetes without clinical evidence of microvascular and macrovascular complications. *Diabetes Care* 2001; **24** (5): 956–7.
45 Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH. Diabetic nephropathy is associated with low-grade inflammation in type 1 diabetic patients. *Diabetologia* 2003; **46** (10): 1402–7.
46 Jialal I, Kaur H. The role of Toll-like receptors in diabetes induced inflammation: implications for vascular complications. *Curr Diab Rep* 2012 Feb 8 (Epub ahead of print).
47 Karlsson Faresjo MG, Ernerudh J, Ludvigsson J. Cytokine profile in children during the first 3 months after the diagnosis of type 1 diabetes. *Scand J Immunol* 2004; **59** (5): 517–26.
48 el-Nawawy A, Soliman T, El-Azzouni O *et al*. Interleukin-1-beta, tumor necrosis factor-alpha, insulin secretion and oral glucose tolerance in non-diabetic siblings of children with IDDM. *Indian J Pediatr* 1998; **65** (3): 455–60.
49 Ozer G, Teker Z, Cetiner S *et al*. Serum IL-1, IL-2, TNF-alpha and INF-gamma levels of patients with type 1 diabetes mellitus and their siblings. *J Pediatr Endocrinol Metab* 2003; **16** (2): 203–10.
50 Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin dependent diabetes mellitus. *Biochem Pharmacol* 1998; **55** (8): 1139–49.
51 Erbagci AB, Tarakcioglu M, Coskun Y, Sivasli E, Sibel Namiduru E. Mediators of inflammation in children with type I diabetes mellitus: cytokines in type I diabetic children. *Clin Biochem* 2001; **34** (8): 645–50.
52 Geerlings SE, Brouwer EC, Van Kessel KC, Gaastra W, Stolk RP, Hoepelman AI. Cytokine secretion is impaired in women with diabetes mellitus. *Eur J Clin Invest* 2000; **30** (11): 995–1001.
53 Netea MG, Hancu N, Blok WL *et al*. Interleukin 1 beta, tumour necrosis factor-alpha and interleukin 1 receptor antagonist in newly diagnosed insulin-dependent diabetes mellitus: comparison to long-standing diabetes and healthy individuals. *Cytokine* 1997; **9** (4): 284–7.
54 Rapoport MJ, Mor A, Vardi P *et al*. Decreased secretion of Th2 cytokines precedes up-regulated and delayed secretion of Th1 cytokines in activated peripheral blood mononuclear cells from patients with insulin-dependent diabetes mellitus. *J Autoimmun* 1998; **11** (6): 635–42.