Prolactin and vitiligo: proposed autocrine/paracrine actions

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Background
The exact cause of vitiligo remains unclear. Several theories have been proposed, including autoimmune and neuroendocrine theories. Prolactin has been studied as an immunomodulatory factor in several autoimmune diseases. Regarding vitiligo, previous studies revealed contradictory results. This is the first study to assess tissue prolactin and prolactin receptor (PRLR) expression in vitiligo.
Objective
To measure the level of serum prolactin, tissue prolactin, as well as PRLR in patients with vitiligo vulgaris and controls to verify their possible role in vitiligo pathogenesis.
Patients and methods
A case–control study was conducted on 40 participants: 20 patients with vitiligo and 20 age-matched and sex-matched healthy controls. Blood samples were taken to determine the serum prolactin level (ng/ml). Skin biopsies were obtained from the lesional skin of patients and normal skin of controls to determine the level of tissue prolactin (ng/mg) and PRLR (ng/g) by enzyme-linked immunosorbent assay.
Results
Serum and tissue prolactin and PRLR levels were significantly higher in patients than in controls ($P<0.001$). Tissue prolactin level had a positive correlation with PRLR level ($r=0.739$, $P<0.001$).
Conclusion
Prolactin plays a role in the pathogenesis of vitiligo, mainly from an intracutaneous position where an autocrine/paracrine loop could have a larger share.

Keywords:
autocrine, paracrine, pathogenesis, prolactin, receptor, vitiligo

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Introduction

Vitiligo is a chronic acquired depigmenting disorder, resulting from the loss of melanocytes from the basal epidermal layer, with a prevalence of ~1% in the world population. It is characterized by milky white macules and patches [1]. The etiology of vitiligo is multifactorial. Several theories have been proposed in a trial to explain this complex disease, including the autoimmune and the neuroendocrine theories [2].

Prolactin is a 199-amino-acid-long polypeptide (23 kD) that acts systemically as a hormone, and locally as a cytokine [3]. It also operates as a part of a neuroendocrine-immune network by stimulating the release of specific cytokines [4]. Prolactin and prolactin receptor (PRLR) expressions have been demonstrated in several cutaneous cell populations, including keratinocytes, fibroblasts, sweat glands, and sebaceous glands [5].

Prolactin has been studied as an immunomodulatory factor in several autoimmune diseases like psoriasis, alopecia areata, and lupus erythematosus [6–8]. Regarding vitiligo, previous studies assessed the serum level of prolactin, and results were contradictory [9–12]. Local production of prolactin was suggested to have a higher correlation with the disease [11], but it was not measured before in the tissue.

In our study, we aimed to measure the serum level of prolactin, tissue prolactin, as well as the expression of its receptors in patients with vitiligo in an attempt to verify their possible roles in the pathogenesis of this disease. The study was conducted on 20 patients with vitiligo vulgaris as well as 20 healthy controls.

Patients and methods

This case–control study was approved by the institution's ethical committee. Informed consents were taken from all participants before enrollment in the study.

Patients

Patients with vitiligo vulgaris above the age of 18 years, not taking any systemic treatment in the last 3 months,

were included in the study. Exclusion criteria included chronic hepatic, renal, thyroid, and autoimmune disorders; epilepsy; pregnancy or lactation; and intake of drugs that affect the serum prolactin level.

Patients were subjected to full history taking, including age; sex; onset, course, and duration of the disease; triggering factors, for example, stress and trauma; history of activity (new lesions, expansion of old lesions, and depigmentation of repigmented areas); and family history of vitiligo or other dermatological diseases. Examination of the skin was carried out to determine the severity of the disease using vitiligo area scoring index (VASI) score and disease activity using vitiligo disease activity (VIDA) score.

Serum sample collection and skin biopsy taking

A 5-ml morning nonfasting blood sample was taken from patients and controls in a plain tube. Avoidance of exercise before sample collection was ascertained. The blood sample was centrifuged at 3000*g* for 10 min, and then serum was taken and stored at temperature −20°C. Thereafter, 4-mm punch biopsies were taken from lesional skin of the patients and healthy skin of controls in empty Eppendorf tubes and stored at temperature −20°C.

Quantitation of prolactin receptor in skin tissue

Each skin biopsy was weighed and homogenized in PBS (400 µl). The homogenate was centrifuged for 10 min at 10 000*g*. The supernatant was separated to be used for quantitation of PRLR level using Human Prolactin Receptor ELISA (Cat. No E4610Hu) that was provided by Bioassay Technology Laboratory (Shanghai, China). This kit is an enzyme-linked immunosorbent assay (ELISA). The plate has been precoated with human PRLR antibody. PRLR present in the sample was added and bound to antibodies coated on the wells. Then biotinylated human PRLR antibody was added and bound to PRLR in the sample. Then streptavidin–horseradish peroxidase (HRP) was added and bound to the biotinylated PRLR antibody. After incubation, unbound streptavidin–HRP was washed away during a washing step. Substrate solution was then added, and color developed in proportion to the amount of human PRLR. The reaction was terminated by addition of acidic stop solution, and absorbance was measured at 450 nm.

Quantitation of prolactin in serum and tissue by enzyme-linked immunosorbent assay kit

The level of prolactin was determined in serum and supernatant of the tissue homogenate using ELISA kit. The Prolactin Quantitative Test Kit is based on a solid-phase ELISA. The assay system used one anti-prolactin antibody for solid-phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (HRP)-conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60 min incubation at room temperature, the wells were washed to remove unbound labeled antibodies. A solution of TMB was added and incubated for 20 min, resulting in the development of a blue color. The color development was stopped with the addition of 2 N HCl, and the color changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

Data management and statistical analysis

Data were coded and entered using the statistical package for the social sciences (SPSS), version 26 (IBM Corp., Armonk, New York, USA). Data were summarized using mean, SD, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the nonparametric Mann–Whitney test. Correlations between quantitative variables were done using the Spearman correlation coefficient. *P* values less than 0.05 were considered as statistically significant.

Results

Demographic and clinical data are summarized in Tables 1 and 2.

Results of levels of serum prolactin, tissue prolactin, and prolactin receptor

On comparing the level of serum prolactin, tissue prolactin, and PRLR in patients versus controls, there were statistically significant differences between the patients and controls, with higher levels in patients than controls ($P<0.001$), as summarized in Table 3.

Table 1 Demographic data of patients and controls

	Vitiligo patients (*N*=20)	Controls (*N*=20)	*P* value
Age (years)			
Range	19–65	22–56	0.2
Mean±SD	34.2±13.5	39.2±10.8	
Sex			
Female/male	10/10	10/10	1

Statistically significant positive correlations were found in the patients' group between serum prolactin and tissue prolactin levels (r=0.614, P=0.004) (Fig. 1), tissue prolactin and prolactin receptor levels (r=0.739, P<0.001) (Fig. 2) while no correlation was found between serum prolactin and prolactin receptor levels (r=0.344, P=0.137).

Table 2 Clinical data of patients

Duration of disease (months)	Range	12–36
	Mean±SD	23.35±8.14
	Median	22
VASI score	Range	0.4–12.5
	Mean±SD	3.99±3.22
	Median	3.10
VIDA score	Range	+1–+4
	Mean±SD	3.2±0.95
	Median	3.50
History of psychic stress (*n*/%)	11/55	
Positive family history (*n*/%)	6/30	

VASI, vitiligo area scoring index; VIDA, vitiligo disease activity.

In controls, the levels of PRLRs showed a significant positive correlation with the levels of serum prolactin (r=0.575, P=0.008) (Fig. 3) but not with tissue prolactin levels (r=0.348, P=0.132). Moreover, serum prolactin did not show a correlation with tissue prolactin levels (r=0.212, P=0.369).

No correlations could be found between serum prolactin level and VASI or VIDA scores (r=0.248, P=0.291, and r=0.059, P=0.803, respectively). Moreover, tissue prolactin level did not show correlations with VASI or VIDA scores (r=–0.199, P=0.401, and r=0.267, P=0.255, respectively). Similarly, no correlations were found between PRLR level and VASI or VIDA scores (r=0.015, P=0.950, and r=0.189, P=0.425, respectively).

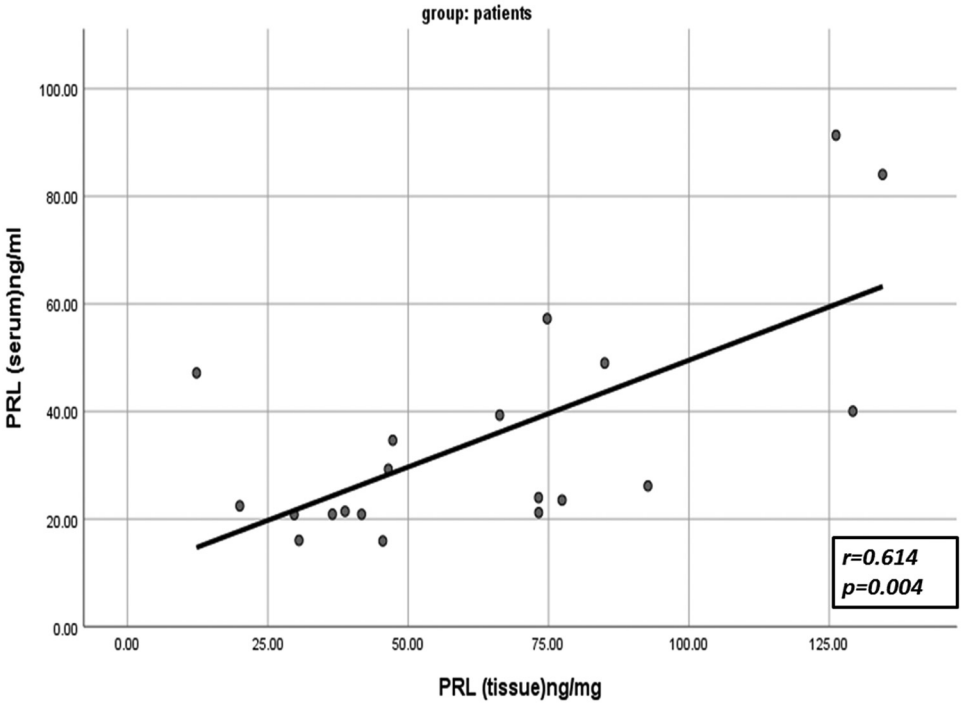
Moreover, no correlations were found between the disease duration and serum prolactin or tissue prolactin or PRLR levels (r=0.187, P=0.43;

Table 3 Summary of serum prolactin, tissue prolactin and prolactin receptors levels in patients and control groups

	Vitiligo patients (N=20)		Control (N=20)		P value
	Range	Mean±SD	Range	Mean±SD	
Serum prolactin (ng/ml)	15.92–91.34	35.27±21.37	4.21–10.45	7.63±2.04	<0.001
Tissue prolactin (ng/mg)	12.3–134.5	64.06±35.95	2.09–12.33	7.53±2.64	<0.001
Prolactin receptor (ng/g)	6.82–93.15	37.62±27.06	0.82–8.15	4.71±2.14	<0.001

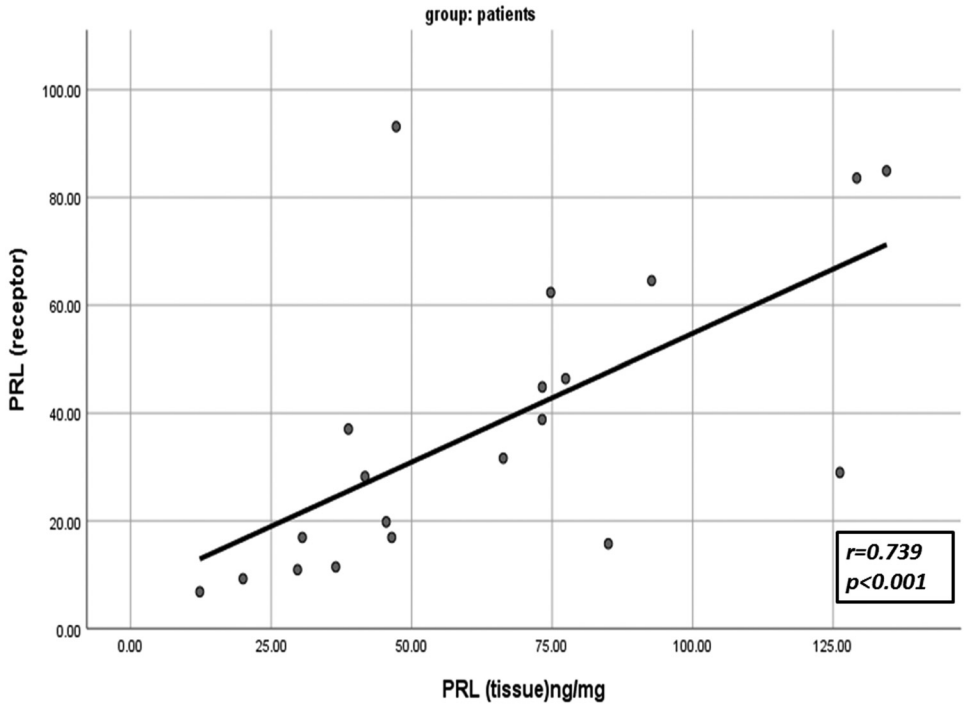
P value less than 0.05 is statistically significant.

Figure 1



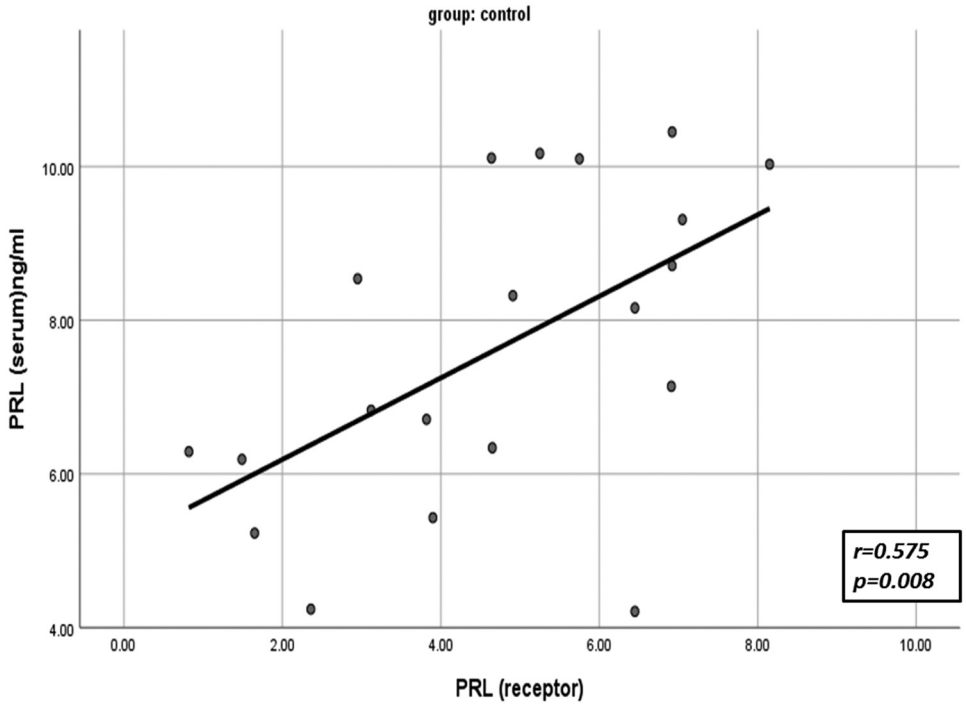
Correlation of serum prolactin level with tissue prolactin level in patients.

Figure 2



Correlation of tissue prolactin level with prolactin receptor level in patients.

Figure 3



Correlation of serum prolactin level with prolactin receptor level in controls.

r=0.063, P=0.792; and r=0.044, P=0.855, respectively).

Regarding the relation with sex and psychic stress, a significant association was found between the serum prolactin level and the sex (higher in females) in both patients and controls (P=0.035, P<0.001, respectively), whereas no significant relation was found between the history of psychic stress and levels of serum prolactin, tissue prolactin, and

PRLR levels (P=0.603, P=0.766, and P=0.766, respectively).

Discussion

Prolactin belongs to the prolactin/growth hormone/placental lactogen family. It is generated and secreted by the lactotroph cells of the anterior pituitary gland [13]. Prolactin has been recognized to exert several physiological functions in addition to its classical role in lactation and reproduction [6].

All recognized PRL functions are thought to be mediated by high-affinity cognate membrane receptors. These high-affinity PRLRs are closely related to the growth hormone receptor and belong to the type I cytokine receptor superfamily. Apart from the pituitary and mammary glands, PRLRs are also expressed in other sites including the skin [14].

In the current study, we have found a significantly higher level of serum prolactin in patients compared with controls. This goes hand in hand with the findings of Elsherif *et al.* [9]. In contrast, Gönül *et al.* [10], Radvar *et al.* [11], and Bozkurt *et al.* [12] found no significant difference in serum prolactin levels between patient and control groups.

Prolactin has multiple immunostimulatory effects and promotes autoimmunity [15], which plays a crucial role in vitiligo. Prolactin promotes the survival, proliferation, and differentiation of T lymphocytes [16]. It also activates protein kinase C, which is required for T-cell proliferation, and increases IFNγ production through interleukin 2 (IL-2) receptor expression and interferon regulatory factor 1, which is important for differentiation of T and B cells. Thus, prolactin stimulates both humoral and cell-mediated immune responses [12]. Antigen-specific CD8+ T cells mediate destruction of melanocytes in vitiligo [17].

In addition, prolactin increases the synthesis of IL-6 and IL-2, which play an important role in melanocytic cytotoxicity in patients with vitiligo [18]. Moreover, prolactin increases the synthesis of tumor necrosis factor alpha (TNF-α) [19], which stimulates nuclear factor kappa B and TNF apoptosis-inducing ligand (TRAIL), which induces melanocyte apoptosis. It also stimulates intercellular adhesion molecule-1 which influences melanocyte recognition by T cells and mediates immunologic cytotoxic damage. TNF alters melanocyte-stimulating hormone receptor and melanocortin-1 receptor, which alter the melanogenesis [20].

To the best of our knowledge, this is the first study to assess tissue PRLR expression in vitiligo. Their levels were significantly higher in patients in comparison with controls. These findings support the hypothesis of local action of prolactin in vitiligo, thus playing autocrine and paracrine roles modifying the local milieu of melanocytes. Autocrine and paracrine actions of tissue prolactin are complementary to the endocrine actions of pituitary prolactin in immune response production and provide mechanisms for the induction of autoimmunity [21].

The regulation of intracutaneous prolactin production is still not completely understood. It is even unknown whether the locally produced prolactin acts in an autocrine/paracrine fashion within the skin only, or does it have a peripheral action? In the current study, we have found a significant correlation between serum and tissue prolactin levels in patients not in healthy controls, with higher levels in tissue than in serum. This can suggest that skin-derived prolactin may diffuse into peripheral circulation, contributing to hyperprolactinemia seen in patients with vitiligo.

Regulation of PRLR expression is a complex process affected by many factors including prolactin level, the status of cell membrane receptors, and the availability of intracellular signaling proteins. Both prolactin-induced upregulation and downregulation of PRLR have been reported in different studies [22]. Our results demonstrated a significant positive correlation between serum prolactin and PRLR expression in healthy controls, denoting a positive feedback regulation. In patients with vitiligo, we found a positive correlation between tissue prolactin rather than serum prolactin and PRLR expression. This may be owing to the local overproduction of prolactin in patients with vitiligo.

Emotional stress can affect, reveal, or even exacerbate several skin disorders, including vitiligo. However, the direct pathophysiologic link between stress factors and cutaneous disease remains unclear [15]. Prolactin is known to be a neuroendocrine mediator of stress response [23]. Stressful life events are known triggers of vitiligo in genetically predisposed individuals. However, stressful life events were not found to be associated with vitiligo extent and distribution [24]. Similarly, we did not find an association between prolactin or PRLR levels and the history of stressful events in our patients.Our finding of a significant association between serum prolactin levels and the sex of patients, where they were higher in females, can be explained by the reciprocal relationship between estrogen and

prolactin. Estrogen is known to stimulate prolactin secretion, whereas high prolactin levels inhibit estrogen secretion. This symphonic interaction between both hormones has numerous effects on the immune system, which leads to a preponderance of autoimmunity in females [25].

Conclusion

In conclusion, prolactin seems to be one of the involved cytokines in the pathophysiological process of vitiligo. We propose that its role is mainly through an intracutaneous action where an autocrine/paracrine loop could have a larger share. Moreover, hyperprolactinemia seen in patients with vitiligo is a reflection of the increased local production rather than a systemic pathological process. Evidence of involvement of PRLR in the pathophysiological reactions opens the door for clinical trials to study the efficacy of different PRLR antagonists, for example, bromocriptine, in the treatment of vitiligo. Large-scale studies on different clinical types of vitiligo are recommended to confirm our findings. Moreover, immunohistochemical studies of the source of skin-derived prolactin in vitiligo are highly recommended.

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Conflicts of interest

There are no conflicts of interest.

References

1 Ezzedine K, Lim HW, Suzuki T, Katayama I, Lan CCE, Goh BK, *et al.* Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res 2012; 25:E1–E3.

2 Arora AK, Kumaran MS. Pathogenesis of vitiligo: an update. Pigment Int 2017; 4:65.

3 Chilton BS, Hewetson A. Prolactin and growth hormone signaling. Curr Top Dev Biol 2005; 68:1–23.

4 Ben-Jonathan N, Hugo ER, Brandebourg TD, LaPensee CR. Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab 2006; 17:110–116.

5 Foitzik K, Krause K, Conrad F, Nakamura M, Funk W, Paus R. Human scalp hair follicles are both a target and a source of prolactin, which serves as an autocrine and/or paracrine promoter of apoptosis-driven hair follicle regression. Am J Pathol 2006; 168:748–756.

6 Lee YH, Song GG. Association between circulating prolactin levels and psoriasis and its correlation with disease severity: a meta-analysis. Clin Exp Dermatol 2017; 43:27–35.

7 El Tahlawi SM, El Eishi NH, Kahhal RK, Hegazy RA, El Hanafy GM, Abdel Hay RM, Shaker OG. Do prolactin and its receptor play a role in alopecia areata?. Indian J Dermatol 2018; 63:241.

8 Jara LJ, Medina G, Saavedra MA, Vera-Lastra O, Torres-Aguilar H, Navarro C, *et al.* Prolactin has a pathogenic role in systemic lupus erythematosus. Immunol Res 2017; 65:512–523.

9 Elsherif NA, El-Sherif AI, El-Dibany SA. Serum prolactin levels in dermatological diseases: a case-control study. J Dermatol Dermatol Surg 2015; 19:104–107.

10 Gönül M, Gül U, Kılınç C, Cakmak SK. Prolactine levels in the patients with vitiligo. J Eur Acad Dermatol Venereol 2009; 23:1454–1455.

11 Radvar SE, Tehranchi Z, Partovikia M, Kazeminejad A, Amini SH. Assessment of prolactin levels in vitiligo patients and healthy controls and its association with severity of disease. Pigment Disord 2014; 2:4172.

12 Bozkurt EÇ, Bozkurt H, Yılmaz A, Atacan D. Serum-prolactin levels in patients with vitiligo. J Clin Anal Med 2019; 10:343–347.

13 Ben-Jonathan N, LaPensee CR, LaPensee EW. What can we learn from rodents about prolactin in humans? Endocr Rev 2008; 29:1–41.

14 Goffin V, Bernichtein S, Touraine P, Kelly PA. Development of potential clinical uses of human prolactin receptor antagonists. Endocr Rev 2005; 26:400–422.

15 De Bellis A, Bizzarro A, Pivonello R, Lombardi G, Bellastella A. Prolactin and autoimmunity. Pituitary 2005; 8:25–30.

16 Carreno PC, Sacedon R, Jimenez E, Vicente A, Zapata AG. Prolactin affects both survival and differentiation of T-cell progenitors. J Neuroimmunol 2005; 160:135–145.

17 Basak PY, Adiloglu AK, Ceyhan AM, Tas T, Akkaya VB. The role of helper and regulatory T cells in the pathogenesis of vitiligo. J Am Acad Dermatol 2009; 60:256–260.

18 Singh S, Singh U, Pandey SS. Serum concentration of IL-6, IL-2, TNF-α, and IFNγ in vitiligo patients. Indian J Dermatol 2012; 57:12.

19 Yang L, Hu Y, Li X, Zhao J, Hou Y. Prolactin modulates the functions of murine spleen CD11c-positive dendritic cells. Int Immunopharmacol 2006; 6:1478–1486.

20 Lemarroy-Camara CR, Alanis-Salas JC. The role of tumor necrosis factor-α in the pathogenesis of vitiligo. Am J Clin Dermatol 2013; 14:343–350.

21 Méndez I, Cariño C, Díaz L. Prolactin in the immunological system: synthesis and biological effects. Rev Investig Clín 2005; 57:447–456.

22 Soboleva TK, Vetharaniam I, Nixon AJ, Montenegro R, Pearson AJ, Sneyd J. Mathematical modelling of prolactin-receptor interaction and the corollary for prolactin receptor gene expression in skin. J Theor Biol 2005; 234:289–298.

23 Torner L. Actions of prolactin in the brain: from physiological adaptations to stress and neurogenesis to psychopathology. Front Endocrinol 2016; 30:7–25.

24 Silverberg JI, Silverberg NB. Vitiligo disease triggers: psychological stressors preceding the onset of disease. Cutis 2015; 95:255–262.

25 McMurray RW. Estrogen, prolactin, and autoimmunity: actions and interactions. Int Immunopharmacol 2001; 1:995–1008.