Prolactin level changes in pemphigus vulgaris: A cohort study

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Background: Prolactin (PRL) appears to play a role in the pathogenesis of autoimmune diseases. Limited evidence showed an association between serum PRL levels and the activity of pemphigus vulgaris (PV). This study intends to determine PRL level changes in pemphigus patients during therapy and its correlation with disease type and severity.

Methods: In this cohort study, we measured serum PRL levels by enzyme-linked immunosorbent assay (ELISA) in newly diagnosed PV patients at three time points: before therapy initiation and after two and four months. Concomitantly, we estimated disease severity by the Pemphigus Disease Area Index (PDAI).

Results: We examined 42 new cases of PV. Among 32 cases who completed the study, mean serum PRL levels at the three time points were 15.9±14.1 ng/mL (before treatment), 16.7±9.8 ng/mL (2 months after initiation of treatment), and 15.2±9.2 ng/mL (4 months after initiation of treatment). Mean PDAI values were 19.3±12.8 (before treatment), 3.7±6.2 (2 months after initiation of treatment), and 0.6±1.5 (4 months after initiation of treatment). Although the disease activity decreased significantly ($P<0.001$), there was no significant change observed in PRL level ($P=0.760$). We observed no significant correlations between PRL levels and PDAI for before ($r= - 0.25$; $P=0.180$), 2 months after initiation of treatment ($r= 0.02$; $P=0.920$), and 2 months after initiation of treatment ($r=0.04$; $P=0.800$).

Conclusion: The results suggest that no significant association exists between serum PRL concentrations and the severity of PV.

Keywords: pemphigus vulgaris, prolactin, pemphigus severity, Pemphigus Disease Area Index

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INTRODUCTION

Prolactin (PRL) appears to have a role in the pathogenesis of autoimmune diseases. It has an important effect on modulating the immune response and promotion of autoimmunity [1]. PRL can be secreted from lymphocytes, enhance the proliferative response of B cells to antigens, and increase the production of autoantibodies. Recently, an association has been proposed between hyperprolactinemia (HPRL) and autoimmune diseases such as systemic lupus erythematosus (SLE), multiple sclerosis, systemic sclerosis, and Sjogren's syndrome. Correlation between PRL level and disease activity has been shown in SLE and rheumatoid arthritis. The potential benefit of dopamine agonists in the treatment of autoimmune diseases is suggested [2,3].

There are limited reports of higher serum PRL levels in pemphigus patients and a positive correlation between PRL level and extent of skin involvement [4,5]. In one case report of a patient

with HPRL and PV, treatment with bromocriptine has been shown to improve skin lesions, after which cessation of therapy caused PV relapse. We conducted a study to measure serum PRL levels in newly diagnosed pemphigus patients before and during treatment to show PRL changes and its correlation with disease type and severity. In the presence of parallel changes, we could suggest the use of this serum marker as a marker for disease activity.

PARTICIPANTS AND METHODS

We conducted this cohort study on PV patients were admitted to the Autoimmune Bullous Diseases Research Center, Razi Hospital, Tehran, Iran from March 2011 through March 2013. The protocol of this study was designed in accordance with the Declaration of Helsinki and implemented after receiving approval from the University Ethics Board.

Eligible patients were new cases of PV diagnosed based on clinical (mucosal and/or cutaneous bullous lesions), histopathologic (suprabasal acantholysis), and direct immunofluorescence (intercellular deposition of IgG and C3 in the epidermis) characteristics, who had not received any systemic treatment for their disease. We excluded patients with any of the following characteristics: current use of medications that cause changes in serum PRL levels, such as antipsychotics (phenothiazines), alpha-methyldopa, phenytoin, and narcotics; pregnancy and lactation; history of abortion; liver failure; renal failure; hypothyroidism; diseases of the hypothalamus; PRL-secreting pituitary adenomas; and trauma to the chest wall. Each participant provided written informed consent for study participation.

Per the study protocol, we measured PRL levels in newly diagnosed PV patients at the start of therapy, and after 2 and 4 months. Participants provided 5 mL of blood samples at these time points. The samples were centrifuged to separate the plasma and stored at –70°C. Then, the samples were transferred to the laboratory to measure PRL levels by enzyme-linked immunosorbent assay (ELISA; Monoband, USA). Due to the lack of sufficient ELISA kits in our center, we used the electrochemiluminescence (ECL) technique (Elecsys analyzers, Roche, Germany) for some of the samples. We converted the microIU/mL to ng/mL by multiplying the measures by 0.047. Normal values for women per the ELISA kit were as follows - under 13 years: 0-10 ng/mL, 13-18 years: 0-51 ng/mL, adults: 1.2-28.5 ng/mL, and menopause: 11.5-18.5 ng/mL. The normal values for males were 5-25 ng/ml for 13 to 18-year-olds and 1.8-20 for others.

We assessed disease severity by the Pemphigus Disease Area Index (PDAI) at the three time points for serum sampling. The patients' treatment protocol included systemic corticosteroid (prednisolone 1-2 mg/kg/d) with or without adjuvant (azathioprine, mycophenolate mofetyl or cyclophosphamide) treatment according to disease severity. Other basic characteristics of patients were recorded on a questionnaire.

We used mean and standard deviation for continuous variables. Pearson and Spearman correlation tests were used to detect the linear relationships between quantitative variables. The independent samples *t* test was employed to assess the differences between means of quantitative variables in the two groups. Repeated measures ANOVA was conducted to determine whether there were statistically significant differences in quantitative variables in a given period of time. The chi-square test was used to detect the relationship between categorical variables. All tests applied were two-sided, with a significance level set at 0.05. All statistical analyses were performed with statistical software IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA).

RESULTS

We examined 42 new cases of PV. The mean age ± SD of patients was 44.3±13.2 years (range: 17–73 years) and there were 16 (38.1%) male patients. A total of 30 (71.4%) had mucocutaneous PV, 9 (21.4%) had pure mucosal PV, and the remaining 3 (7.1%) had pure skin type PV.

We measured serum PRL levels at 2 months after the first measurement in 36 patients (6 patients were missed) and in 32 patients at 4 months (4 additional patients were missed). There was no statistically significant difference observed in the demographic analysis of the 6 missing patients in the month 2 measurement compared to the remaining patients in terms of age (P=0.220), sex (P=0.380), pemphigus severity (P=0.420) and type

(*P*=0.760). The month 4 measurement also had the same findings for age (*P*=0.499), sex (*P*=0.270), pemphigus severity (*P*=0.170), and type (*P*=0.295).

The serum PRL levels did not significantly differ at the different time points during treatment. PDAI decreased significantly during this period of time. There was no significant correlation found between PDAI and PRL at the beginning, 2- and 4-month time points (Table 1).

At diagnosis, we measured serum anti-desmoglein 1 and 3 (anti-Dsg1 and 3) antibodies as another estimation of disease severity. PDAI before treatment (PDAI1) showed a strong positive correlation with anti-Dsg1 (*r*=0.62, *P*=0.001) and anti-Dsg3 (*r*=0.61, *P*=0.002). No significant correlation existed between PRL1 and anti-Dsg1 (*r*=-0.20, *P*=0.260) and anti-Dsg3 (*r*=-0.18, *P*=0.320).

PRL level did not show any significant correlation with the age of the patients at diagnosis (*r*= - 0.27; *P*=0.090), 2 months (r= 0.09; *P*=0.590), and 4 months (*r*=0.27; *P*=0.110) after treatment. We observed the same results when the analysis was separately performed for males at diagnosis (*r*=-0.34; *P*=0.21), 2 months (*r*=0.11; *P*=0.690), and 4 months (*r*=0.17; *P*=0.560) compared to females at diagnosis (*r*=-0.29; *P*=0.160), 2 months (*r*=0.09; *P*=0.690), and 4 months (*r*=0.35; *P*=0.110) after treatment. No significant association existed between PRL levels and gender at diagnosis (*P*=0.06), 2 months (*P*=0.590), and 4 months (*P*=0.690). Serum PRL changes did not differ at different time points between male and female patients after adjustments for age (*P*=0.230; Figure 1). The same result was shown for pemphigus type (*P*=0.440).

A total of 6 out of 42 patients (14.3%) had HPRL at their first visit. At 2 months after treatment, 19% had HPRL and 4 months after treatment, 14.3% of patients had HPRL. However, different patients had HPRL at each visit. From the 5 patients with HPRL at the first visit that were seen at 2 months, only one had HPRL at 2 months. Patients with HPRL did not differ from other patients in terms of age, gender, and pemphigus type. Serum PRL levels did not change significantly in patients with HPRL at the first visit PRL1=38.3±21.8 at the first visit, PRL=21.9±5.1 after 2 months, PRL=21.3±9.4; 4 months later; *P*= 0.14). There was an insignificant decrease in PDAI (PDAI=8.0±5.5 at the first visit, PDAI=0.5±1.0 after 2 month, PDAI=0.8±0.9 4 months later; *P*=0.081) in this group.

Patients that had HPRL at diagnosis reported a mean PDAI score of 8, which was lower than the remainder of patients (mean PDAI= 20.9), however the difference was not statistically significant (*P*=0.057). Table 2 shows the mean serum PRL levels and severity indices of patients with or without HPRL at the three time points. Anti Dsg-1 and 3 before treatment did not differ between patients with or without HPRL.

DISCUSSION

In this study, we measured serum PRL levels in PV patients at the start of treatment, and 2 and 4 months after treatment initiation. We did not find any correlation between PRL level with disease

Table 1. Serum prolactin and PDAI in pemphigus vulgaris patients at diagnosis and 2- and 4-month after treatment.

	At diagnosis	2 months after treatment	4 months after treatment	*P*
PRL (ng/ml)	15.9±14.1	16.7±9.8	15.2±9.2	0.760
PDAI	19.3±12.8	3.7±6.2	0.6±1.5	<0.001
Pearson correlation coefficient	*r*= -0.25 *P*=0.18	r=0.02 *P*=0.92	*r*=0.04 *P*=0.80	

PRL: Prolactin; PDAI: Pemphigus Disease Area Index

Table 2. Comparison of serum prolactin and PDAI between pemphigus vulgaris patients with or without hyperprolactinemia.

Time of measurement		HPRL patients	Non-HPRL patients	*P*
At diagnosis	Mean PRL (ng/ml)	42.6±22.2 (n=6)	11.5±6.2 (n=36)	0.018
	Mean PDAI	8±5.5 (n=4)	20.9±12.7 (n=27)	0.057
2 months after treatment	Mean PRL (ng/ml)	28.9±9.7 (n=8)	12.8±5.8 (n=28)	<0.001
	Mean PDAI	2.1±3.3 (n=8)	3.5±6.3 (n=28)	0.570
4 months after treatment	Mean PRL (ng/ml)	29.9±4.8 (n=6)	11.5±5.6 (n=31)	<0.001
	Mean PDAI	0.5±0.8 (n=6)	1.4±4.5 (n=31)	0.646

HPRL: Hyperprolactenemia , PRL: prolactin, PDAI: Pemphigus Disease Area Index.

severity and serum anti-Dsg1 and 3 antibodies. To the best of our knowledge, this was the first study that evaluated serum PRL levels at different time points during the course of PV and the effect of treatment on serum PRL. HPRL had an incidence of 14.3% to 19% in PV patients at different measurements during their treatment period. This result supported findings of another study that compared 24 PV patients to 24 healthy age- and sex-matched individuals [5]. In this study, 25% of patients and none from the control group had HPRL. Interestingly, the patients with HPRL in the current study differed at different measurements. The prevalence of HPRL in a healthy population has been reported to be 0.4% to 3% [2]; pemphigus patients may show HPRL during their disease course, but this elevation is not persistent.

Khandpur and Reddy [4] reported a strong association between the severity of PV and serum PRL levels in a 21-year-old woman with idiopathic HPRL and extensive mucocutaneous pemphigus. Her skin lesions improved after treatment with bromocriptine and relapsed after the cessation of therapy. Fallahzadeh, *et al.* observed higher serum PRL levels in patients with more extensive skin involvement (P<0.01). This finding did not correlate with disease type (cutaneous, mucosal or mucocutaneous) [5]. Conversely, according to our study, patients with HPRL at the first visit had less severe disease with a PDAI score of 8 versus 20.9. This difference was not statistically significant. Although disease severity scores (PDAI) decreased significantly after treatment, mean serum PRL levels in patients did not change. Therefore, we could conclude that treatment of pemphigus (4 months) did not elicit a statistically significant reduction in PRL levels. The discrepancy between these results might be explained by using different severity measurement scales. They have calculated the involved body surface area using the Lund and Browder chart, which is used to estimate skin surface involvement in burns but does not consider mucosal lesions. In the current study, we have used the PDAI that estimates PV severity, including mucosal lesions. The difference could also be explained by circadian variations in serum PRL levels and sample size limitation. Therefore, PRL levels might be correlated with only skin lesions, not overall PV severity (as measured by the current research).

Our data supported the association of pemphigus severity with anti-Dsg1 and 3 antibodies. Another study showed that severity of skin lesions, but not oral lesions, correlated with anti-Dsg1 antibody. Anti-Dsg3 antibody correlated with severity of both skin and oral lesions [6]. PRL levels did not have any correlation with anti-Dsg1 and 3 antibodies, which again showed that serum PRL levels could not be a good marker of disease severity in PV patients at diagnosis.

Other experiments on immune mediated inflammatory diseases such as psoriasis and SLE have indicated that PRL plays a role in the disease process. In a case-control study of psoriasis patients, PRL significantly decreased after treatment. However, no correlation existed between PRL levels and improved PASI score [7]. It has been proposed that HPRL might have a role in the hyperproliferation of keratinocytes in psoriasis [2]. In SLE, studies showed inconsistent results for correlation between disease activity and PRL levels [2]. According to research, bioactive PRL might have a correlation with lupus activity while macroprolactinemia or idiopathic HPRL show a negative association [8]. We observed a negative correlation of idiopathic HPRL with pemphigus activity in the current study, although this was not a statistically significant finding.

The limitations of our study have included small sample size and short follow up duration. We suggest that similar studies focus on treatment interventions for hyperprolactinemic patients in order to determine if PRL lowering agents could affect disease activity. In addition, serum and cutaneous PRL level should be assessed concomitantly. Bioactive PRL concentrations must be measured to determine the presence, if any, of a relationship with disease activity.

In conclusion, the frequency of HPRL appeared to be higher in PV patients, however we observed no significant correlation between serum PRL levels and disease severity. In addition, we could not show any significant relationship between initial PRL levels and serum anti-Dsg antibodies. Treatment of PV had no effect on PRL levels. Therefore, serum PRL levels did not appear to be a good marker of disease severity and response to treatment. Perhaps this was due to numerous factors that affect serum PRL levels. Eating, exercise, various medications, and emotional stress can effect PRL levels. Thus, an abnormal serum PRL level is not

specific and may not be suggested as a routine test for PV patients.

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