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Serologic Biomarkers in Pemphigus Monitoring: C-reactive Protein, Macrophage Migration Inhibitory Factor, and Prolactin Levels Versus Autoantibody Assays

Hossein Mortazavi[1], Vahide Saeidi[1], Kamran Balighi[1], Nafiseh Esmaili[1,2], Amir Teimourpour[3], Maryam Daneshpazhooh[1,2], Shahin Hamzelou[1,2], Zahra Saffarian[4], and Jafar Taghizadeh Fazli[5]

[1] Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2] Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4] Department of Dermatology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[5] Department of Dermatopathology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Evaluation and monitoring of pemphigus vulgaris (PV) typically involve autoantibody detection by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF). We aimed to determine the levels of antipemphigus immunoglobulin (Ig) G autoantibodies using ELISA and IIF (as standard biomarkers), and compare it to prolactin, macrophage migration inhibitory factor (MIF), and C-reactive protein (CRP) (as nonstandard biomarkers) to determine which of these non-standard biomarkers is appropriate for PV monitoring. The experiment was performed before and during therapy.

Anti-Dsg immunoglobulin G autoantibodies were measured using ELISA and IIF (as standard biomarkers) versus prolactin, MIF, and CRP (nonstandard), before 1 and 3 months after the treatment. Before beginning the treatment, the severity of the disease was determined using the pemphigus disease area Index (PDAI). We enrolled 60 newly diagnosed patients with PV (32 men and 28 women; mean age=43.8±14.2 years).

Before treatment, the levels of anti-Dsg1, anti-Dsg3, and IIF were high and had a significant relationship with PDAI. PDAI also had a connection with the levels of CRP and prolactin. The anti-Dsg1, anti-Dsg3, IIF, and CRP titers decreased in patients treated with conventional (prednisolone plus azathioprine) and rituximab therapy during and after treatment.

In conclusion, anti-Dsg1, anti-Dsg3, and IIF autoantibody titers remain standard biomarkers for assessing disease activity, severity, and PV monitoring. The trend of CRP was similar to that of anti-Dsg1, anti-Dsg3, and IIF. Thus, CRP may be used for PV monitoring.

Keywords: Autoantibody; C-reactive protein; Desmogleins; Macrophage migration inhibitory factor; Pemphigus; Prolactin

Corresponding Author: Shahin Hamzelou, MD; Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 912) 139 3128, Fax: (+98 21) 6659 5911, E-mail: dr.hamzelou@gmail.com

INTRODUCTION

Pemphigus vulgaris (PV) is a rare autoimmune blistering disease affecting the skin and mucous

membranes. It can cause severe morbidity and mortality if left untreated. Therefore, early diagnosis and regular monitoring of PV are essential for the optimal management of the disease. Previous studies have shown that anti-desmoglein (anti-Dsg) enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) levels correlate well with each other and with PV severity and activity.[1-3] Avgerinou et al, demonstrated that anti-Dsg1 and anti-Dsg3 antibodies correlate with IIF values and that these tests can be used to monitor PV.[4] Our research group has also investigated the relationship between PV activity and IIF titers.[5]

There is currently no simple and inexpensive test for the evaluation and follow-up of PV in most laboratories in regions like Iran, where the disease is more prevalent.[6] Several studies have proposed new biomarkers,[7-12] such as macrophage migration inhibitory factor (MIF), prolactin (PRL), and C-reactive protein (CRP) in patients with PV, and suggested their potential use as alternative or complementary biomarkers for evaluating PV.[7-9] Furthermore, a correlation has been demonstrated between PV severity and CRP using a semiqualitative CRP measurement.[9] Moreover, in a study conducted on dogs with pemphigus foliaceus, CRP levels decreased as the disease improved.[10]

In this paper, anti-Dsg ELISA and IIF are referred to as standard biomarkers for evaluating PV, while MIF, PRL, and CRP are considered nonstandard biomarkers.

This study aimed to determine standard and nonstandard biomarker levels before, and 1 and 3 months after treatment. We then compared standard and nonstandard biomarkers during the course of PV and evaluated whether nonstandard biomarkers are suitable for monitoring and follow-up of PV patients.

MATERIALS AND METHODS

We enrolled 60 newly diagnosed PV patients who were admitted to Razi Hospital in Tehran, Iran (affiliated with Tehran University of Medical Sciences), between 2016 and 2017. The inclusion criteria were as follows: newly diagnosed untreated patients with PV, diagnosed on the basis of clinical, histopathological, and direct immunofluorescence findings. We excluded patients with a history of immunosuppressive therapy, pregnancy, abortion, lactation, chest wall trauma, renal or hepatic insufficiency, hypothyroidism, chronic inflammatory diseases, and those who had used drugs that increase PRL levels, such as opiates, phenytoin, methyldopa, and antipsychotics (e.g., phenothiazine).

At the initial visit, the severity of the disease was assessed using the Pemphigus Disease Area Index (PDAI). Mucosal, cutaneous, mucocutaneous, and total PDAI were calculated according to the International Pemphigus Study Group recommendations.[13]

Serum biomarker levels of anti-Dsg1, anti-Dsg3, IIF, CRP, MIF, and PRL were measured at 3-time points: before (first visit), 1 month after (second visit), and 3 months after (third visit) treatment.

Anti-Dsg1 and anti-Dsg3 were measured using an ELISA kit (EUROIMMUN AG, Luebeck, Germany) according to the manufacturer's instructions. We used 1:100 diluted serum. Cutoff values for anti-Dsg1 and anti-Dsg3 were 20 relative units per milliliter (RU/mL).

We used a diluted serum on human skin as a substrate for the IIF procedure. The serum was diluted serially with phosphate-buffered saline (PBS). We considered the test positive if the autoantibody was detected in the serum diluted at a 1:10 dilution or higher.

Serum MIF levels were measured using the SEA698Hu 96 Tests ELISA kit (Cloud-Clone Corp., Houston, USA), and the results were expressed as nanograms per milliliter.

Chemiluminescence immunoassay was used to measure serum PRL concentrations (Diasorin kit, Piedmont, Italy). Levels above 15.2 ng/mL in men and 23.3 ng/mL in women are commonly considered elevated and may indicate hyperprolactinemia.[14]

We used a sensitive and accurate automated immunoturbidimetric assay (high-sensitivity CRP, quantitative immunoturbidimetric assay, Pars Azmun kit, Karaj, Iran) to measure CRP levels in the serum. This method was used to detect minor increases in CRP titers. Changes between 3 and 10 mg/L were considered minor elevations, whereas alterations of>10 were considered elevated.[15]

Based on the attending physician's decision and routine PV therapy guidelines, conventional therapy, including prednisolone (1 mg/kg/day) plus azathioprine (2.5 mg/kg/day) or mycophenolate mofetil (2000 mg/day), was used for 50 patients.[16] Rituximab and prednisolone were used in 9 patients who were considered medically suitable for rituximab (i.e., clinical evaluations by a dermatologist, internist, and cardiologist, and check-ups for hepatitis B and C, as well as QuantiFERON and purified protein derivative test) and financially able to afford the drug. Finally, intravenous immunoglobulin (IVIG) was used in 1 patient who was refractory to other treatments.[16]

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The same dermatologist conducted a 3-month clinical follow-up with all patients. All study participants signed an informed consent form.

Statistical Analysis

Spearman's rank correlation coefficient was used to determine the correlation between standard and nonstandard biomarkers and PV severity (PDAI). We used Friedman test with pairwise comparison by Bonferroni adjustment to determine the trend in biomarker changes during the study period. SPSS software (version 21) was used to analyze the data, and *p* values <0.05 were considered significant.

RESULTS

Patient Characteristics

The study enrolled 60 patients with PV, including 32 (53.3%) men and 28 (46.7%) women. The mean±SD age of the patients was 43.8±14.2 years (range: 23–90). The median (interquartile range [IQR]) PDAI values for mucosal, cutaneous, and the entire study population were 17.97 (14.7), 15.58 (12.9), and 33.57 (17.56), respectively (Supplementary Table).

Supplementary Table lists the serum levels of standard (anti-Dsg1, anti-Dsg3, and IIF) and nonstandard (MIF, PRL, and CRP) biomarkers in the total study population before treatment. Twelve patients (5 women and 7 men) exhibited hyperprolactinemia.

Table 1 shows the correlation coefficients and *p* of biomarkers with PDAI in patients with PV before therapy.

Treatment of Patients and Trends in Biomarker Changes

Table 2 shows the levels of biomarkers in 50 patients treated with conventional regimens (prednisolone plus mycophenolate mofetil or prednisolone plus azathioprine) and 9 patients treated with a rituximab regimen. One patient received IVIG.

The supplementary Figure illustrates the decreasing trends in anti-Dsg1, anti-Dsg3, IIF, and CRP levels, based on 3 measurements of mean ranks in patients who received conventional and rituximab therapy.

Based on clinical judgments and assessments, all patients exhibited 85% to 90% improvement in their second and third visits.

Table 1. Correlation coefficients and *p* values of biomarkers with Pemphigus Disease Area Index (PDAI) in patients with pemphigus vulgaris before therapy

Biomarker	PDAI	Correlation Coefficient	*p*
Dsg1	Mucosal	-0.05	0.686
	Cutaneous PDAI	0.785	<0.001
	Total PDAI	0.453	<0.001
Dsg3	Mucosal PDAI	0.315	0.015
	Cutaneous PDAI	-0.03	0.808
	Total PDAI	0.15	0.258
CRP	Mucosal PDAI	0.261	0.046
	Cutaneous PDAI	-0.057	0.666
	Total PDAI	0.187	0.156
PRL	Mucosal PDAI	0.151	0.253
	Cutaneous PDAI	0.195	0.140
	Total PDAI	0.263	0.044
IIF	Mucosal PDAI	0.405	0.001
	Cutaneous PDAI	0.039	0.772
	Total PDAI	0.363	0.005
	Anti-Dsg3	0.392	0.002

Dsg, desmoglein; CRP, C-reactive protein; PRL, prolactin; IIF, indirect immunofluorescence; PDAI, Pemphigus Disease Area Index

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Table 2. Changes in biomarkers in patients with pemphigus vulgaris during conventional and rituximab therapy

	Dsg1 (RU)			Dsg3 (RU)			IIF (*)			CRP (mg/L)			MIF (pg/mL)			PRL (ng/mL)		
50 patients with conventional therapy																		
Measurement time point†	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Median	99.6	35.5	15.9	200	181.4	64.5	320	160	30	2.34	0.6	0.85	1.42	1.3	1.45	9.9	14.9	13.98
Mean Rank	2.84	1.78	1.39	2.48	2.01	1.51	2.88	1.94	1.19	2.58	1.75	1.68	2.05	1.84	2.11	1.7	2.08	2.23
***p* value**	<0.0001			<0.0001			<0.0001			<0.0001			0.419			0.054		
1-2	<0.0001			0.003			<0.0001			<0.0001			1.00			0.021		
1-3	<0.0001			<0.0001			<0.0001			0.0002			1.00			0.018		
2-3	0.012			0.003			<0.0001			0.957			1.00			0.66		
9 patients with rituximab therapy																		
Measurement time point†	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Median	153.3	25.3	2.85	196.4	184.4	76.4	320	160	45	0.77	9.9	0.56	1.4	0.99	1.15	8.1	13.1	12.8
Mean Rank	3	1.75	1.25	2.63	2.38	1	2.88	1.88	1.25	2.22	2.06	1.72	2.25	1.75	2	1.5	2.25	2.25
***p* value**	0.039			0.038			0.037			0.529			0.779			0.472		
1-2	0.084			0.414			0.129			1.00			1.00			0.138		
1-3	0.204			0.204			0.204			0.706			1.00			0.432		
2-3	1.00			0.204			0.54			0.896			1.00			1.00		

†1, before therapy; 2, one month after therapy; and 3, three months after therapy; *dilution of serum in which the test was positive; Dsg, desmoglein; IIF, indirect immunofluorescence; CRP, C-reactive protein; MIF, macrophage migration inhibitory factor; PRL, prolactin; RU, relative units, ml: milliliter

DISCUSSION

In this study, we compared the levels of MIF, PRL, and CRP (as nonstandard biomarkers) for the follow-up of PV patients, with the levels of standard biomarkers to determine which of these nonstandard biomarkers is suitable for PV monitoring.

Before treatment standard biomarkers, namely anti-Dsg1, anti-Dsg3, ELISA, and IIF, are positive and usually found at high levels in untreated PV patients during the active phase of the disease.[5,17]

The median (IQR) values of anti-Dsg1 and anti-Dsg3 had high levels similar to those found in our previous studies; these values were also related to disease activity and severity.[1,2] Anti-Dsg1 antibody levels were significantly correlated with cutaneous PDAI. In addition, mucosal PDAI was significantly correlated with anti-Dsg3 antibody levels, similar to our previous findings.[18]

IIF had relatively high median (IQR) titers in untreated PV patients. Furthermore, the values of total PDAI, mucosal PDAI, and anti-Dsg3 antibodies were significantly correlated with IIF titers. Previously, IIF titers were found to be correlated with the severity and activity of PV.[5,19] Similarly to our study, Barnadas et al, also showed a relationship between IIF titers, mucosal disease, and anti-Dsg3 antibody titers.[20] In a study on dogs with PF, there was a weak correlation between IIF titers and CRP levels.[10]

During treatment significantly decreasing trends in anti-Dsg1, anti-Dsg3, and IIF titers were observed in PV patients with conventional therapy, indicating that these tests (ELISA and IIF) are valuable for following up and monitoring PV patients. Similar conclusions have been drawn previously.[4,21] Additionally, these biomarkers showed a decreasing trend in patients on rituximab therapy. Similarly, reductions in anti-Dsg1 and anti-Dsg3 titers in PV patients on rituximab therapy were reported in another study.[22]

CRP levels higher than 10 mg/L are commonly considered elevated.[15] In our study, we found that the mean level of CRP in the total study population was elevated (10.11 mg/L) before treatment. We also found a significant positive correlation (p=0.261, p<0.046) between mucosal PDAI and CRP in untreated patients. This indicates that CRP reflects disease severity in PV. Our findings are consistent with previous studies that reported a relationship between acantholysis, PV severity and CRP concentration in saliva,[23] or serum.[9]

Moreover, we observed that CRP levels decreased during therapy in both conventional and rituximab groups. This suggests that CRP can be used as a marker for monitoring PV therapy response, especially in areas without ELISA and IIF facilities. In an animal study, in dogs with pemphigus foliaceus, CRP levels decreased during the course of the disease improvement.[10]

Another study has reported a decrease in IL-6 during pemphigus therapy.[24] Since IL-6 measurement is not a routinely available test, its measurement is not suitable for monitoring pemphigus treatment. In addition, IL-6 itself induces CRP production in the liver.[25]

MIF levels were not correlated with disease activity or therapy response in our study. The mean MIF level before therapy was 1.48 pg/mL, which is lower than the MIF level of 11.99 pg/mL previously mentioned by Namazi et al.[7] However, we used a different kit, which may have affected the comparability of our results. No significant trend in MIF levels emerged during the present study. Therefore, we conclude that MIF is not a reliable biomarker of disease activity or therapy response in PV.

On the other hand, PRL levels showed a different pattern. In our study, only 20% of PV patients had hyperprolactinemia before therapy, which is similar to the 22% reported by Lajevardi et al.[14] We also found a significant positive correlation between total PDAI and PRL levels before therapy, indicating that PRL reflects disease severity in PV. Our findings are consistent with those of Yousefi et al,[8] who reported higher values in newly diagnosed pemphigus patients and detected a relationship between PRL levels and disease severity. Conversely, some studies have failed to find any association between PRL and anti-Dsg levels or disease severity.[11,26]

PRL, MIF, and IL-6 may also contribute to the pathogenesis of PV.[7,8,24] Our study has some limitations that need to be acknowledged. PDAI was measured only before starting the treatment; improvements in patients during the second and third visits were evaluated clinically. Also, there was no control group for MIF measurement before starting therapy.

The present study confirmed that anti-Dsg3 and anti-Dsg1, as well as IIF autoantibodies, remain standard biomarkers for the follow-up of PV.[12,21] CRP and PRL were significantly correlated with PDAI in newly diagnosed, untreated PV patients. However, PRL and MIF were not suitable biomarkers for the follow-up of PV

patients. CRP showed a decreasing trend during PV therapy and was correlated with disease improvement. Quantitative measurement of CRP during treatment is recommended. Finally, we conclude that CRP can be a reliable marker of disease activity and therapy response in PV.

STATEMENT OF ETHICS

The project was approved by the Ethics Committee of Tehran University of Medical Sciences (Ethics Committee approval code: IR.TUMS.REC.1395.2565).

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CONFLICT OF INTEREST

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