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Immunological markers as predictors of developing steroid-induced diabetes mellitus in pemphigus vulgaris patients

An observational study

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Abstract

The aim of this study was to evaluate the clinical importance of autoantibodies in pemphigus vulgaris patients who developed steroid-induced diabetes mellitus (SID) because of the glucocorticoid therapy of pemphigus.

A total of 137 patients with pemphigus vulgaris were studied. Patients with SID and pemphigus were compared with those that had only pemphigus. The variables recorded were: age at diagnosis, sex, body mass index, presence of diabetes mellitus (DM), cumulative cortisone dose, treatment duration, value of anti-desmoglein 1 and 3, and anti-glutamic acid decarboxylase autoantibodies.

A total of 31 patients (22.62%) that developed steroid-induced DM were identified. Anti-glutamic acid decarboxylase autoantibodies were positive in 20.75% of patients with pemphigus vulgaris and in 25.75% of patients with pemphigus vulgaris and SID.

The overall anti-glutamic acid decarboxylase autoantibodies prevalence in pemphigus patients was high, and the risk of developing DM in patients with pemphigus remains a serious problem, being associated with increased risk of mortality.

Abbreviations: BMI = body mass index, DM = diabetes mellitus, GADA = glutamic acid decarboxylase autoantibodies, IQR = interquartile ranges, SD = standard deviations, SID = steroid-induced diabetes.

Keywords: anti-glutamic acid decarboxylase autoantibodies, desmogleins, immunology, pemphigus, steroid-induced diabetes mellitus

1. Introduction

Pemphigus comprises a group of autoimmune blistering diseases, immunologically characterized by autoantibody production

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against the antigens of the intercellular junctions of keratinocytes, including the clinically relevant cadherins desmoglein 1 and desmoglein 3.[1]

Patients with pemphigus have increased risk for additional comorbidities with an immune or inflammatory-mediated pathogenesis,[2] such as thyroid disease, rheumatoid arthritis, diabetes mellitus (DM), hypertension, heart disease, and asthma.[3] Different autoantibodies were found in the serum of pemfigus patients directed against thyroglobulin, cardiolipin (IgM), reticulin (IgG), and gliadin (IgG).[2] Islet cell autoantibodies and glutamic acid decarboxylase autoantibodies (GADA) are markers of the autoimmune form of β cell damage, and are present at onset in 70% to 80% of patients with type 1 DM. Their presence was also observed in type 2 DM patients.[4]

Previous studies described an immunological association between type 1 DM and pemphigus.[5,6] Therefore, it is interesting to evaluate GADA prevalence in pemphigus patients, and to assess a possible correlation between these antibodies and the steroid-induced DM (SID). Pemphigus therapy requires chronic administration of combined corticosteroids and immunosuppressive agents. The dose and duration of corticosteroid treatment relates to the clinical severity of the disease and varies from one patient to another.[7]

The aim of the presenty study was to evaluate the incidence of SID in pemphigus vulgaris patients who received glucocorticoid treatment and to characterize SID patients in terms of age, body mass index (BMI), cumulative cortisone dose, treatment duration, and immunological markers (desmogleins 1, 3 and GADA).

Moreover, the study evaluates GADA as a possible immunological marker for the risk of developing DM in pemphigus patients.

2. Materials and methods

2.1. Patients

We performed a retrospective, hospital-based cohort study. The study included a series of 137 patients (age 15–83 years, 82 females and 55 males) with pemphigus vulgaris, who were diagnosed in our department between 2001 and 2015.

Diagnosis of pemphigus was based on clinical and immuno-histochemical criteria.[8] Cutoff value for positive levels of anti-desmoglein 3 and anti-desmoglein 1 was ≥20 U/mL.

All patients received corticosteroid therapy (prednisone) after hospitalization. The average dose was between 0.5 and 1 mg/kg daily, for a period of approximately 1 month, followed by a gradual dose reduction of medication. Median treatment duration was between 6 to 12 months.

SID was diagnosed when the following criteria were met: blood glucose level ≥126 mg/dL, random blood glucose ≥200 mg/dL, HbA1c >6.5%, or blood glucose >200 mg/dL 2 hours after an 75 g oral glucose load test. These criteria are based on the current criteria established by the American Association of Diabetes.[9]

Blood glucose level was determined at admission, before the initiation of steroid therapy, and at subsequent hospitalizations. GADA was measured using enzyme-linked immunosorbent assay kit (Biomerica Inc). The cutoff value for positive levels of GADA was ≥1.05 U/mL.

The study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1983. All subjects received detailed information and signed a written informed consent. The study was approved by the Ethical Committee of University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca. Recorded parameters were: age at diagnosis, sex, presence of DM, value of anti-desmoglein 1 and 3, and GADA. For patients with comorbidity, pemphigus and SID, cumulative cortisone dose, treatment duration, and BMI were determined.

2.2. Statistical analysis

Categorical data were presented as counts and percentages, normally distributed continuous data were presented as means and standard deviations (SD), whereas skewed continuous data were presented as medians and interquartile ranges or median and range. Whether data followed a normal distribution was checked with Kolmogorov-Smirnov test and quantile-quantile plots. The comparison between 2 independent groups regarding normally distributed continuous variables was made with t test for independent samples, or with Wilcoxon rank-sum test regarding skewed data. All statistical tests used the 2-tailed P value, and a significance level of 0.05. Statistical analysis was performed with R environment for statistical computing and graphics, version 3.2.3 [10] and the package Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, www.graphpad.com was used for graphic data representations.

3. Results

A total of 137 patients with pemphigus vulgaris were included in the study. All patients received corticosteroids. The overall demographic characteristics and antibody levels are included in Table 1. Thirty-one (22.62%) patients with new-onset hyperglycemia (SID), 6 (4.37%) patients with type 2 DM, and 4 (2.91%) patients with type 1 DM were identified.

The treatment duration between treatment initiation and the occurrence of diabetes was between 0.25 and 108 months, with a median value of 6 months, and a mean value of 25 months.

We found no correlation between steroid dosage and occurrence of diabetes.

In SID patients, there was no statistically significant difference between sex groups as seen in Figure 1, despite some differences especially for the age and cortisone therapy, for example, doses and duration of therapy that showed lower overall values for males.

Comparison of the immunological markers (positivity and amount) shown in Figure 2 between patients with pemphigus without DM and patients with pemphigus and DM yielded no statistical relevance.

GADA was found positive in 20.75% of patients with pemphigus vulgaris and in 25.75% of patients with pemphigus vulgaris and SID, yet the difference showed no statistical significance.

Overall anti-desmoglein 3 antibodies were positive in the majority of subjects followed by anti-desmoglein 1 antibodies in about 75%, whereas GADA were positive in almost 20% of the subjects.

4. Discussion

In our study, 31 of 137 patients with pemphigus vulgaris (22.62%) developed SID after corticosteroid treatment.

In one study, Turner et al showed that the presence of islet cell antibodies and GADA in patients with type 2 DM suggested an increased likelihood that insulin therapy would be required. Eighty-four percent of patients aged 34 or younger, GADA-positive, required insulin therapy after 6 years. Only 34% of patients older than 55 years and GADA-positive required insulin therapy within 6 years.[4] In our study, the new-onset hyperglycemia persisted even after the treatment with glucocorticoids was stopped. The patients received oral hypoglycemic drugs, and few of them, insulin, with glycemic control. Persistent hyperglycemia could be explained by the status of immunosuppression (induced by pemphigus or the associated treatment with azathioprine). There was no difference between GADA-positive versus GADA-negative pemphigus patients regarding the insulin doses or the control of glycemic values. A possible explanation

Table 1
Overall characterization of the pemphigus patients group,.

Group	Age, y	Sex, male	Dsg 1		Dsg 3		GADA	
			n+	U/mL	n+	U/mL	n+	U/mL
n=137	52.87	n=55	98	104	130	173	30	0.83
	±14.56	40.15%	71.5%	14.9 - 176	94.89%	103- 277	21.9%	0.7- 1.01

Asessment of pemphigus antibodies: Dsg 1, Dsg 3, and GADA are presented as the number (n+) and % of positive patients (left) and quantitative enzyme-linked immunosorbent assay results of antibody levels in U/ml as median/interquartile range (right). Dsg=desmoglein, GADA=glutamic acid decarboxylase; n=number.
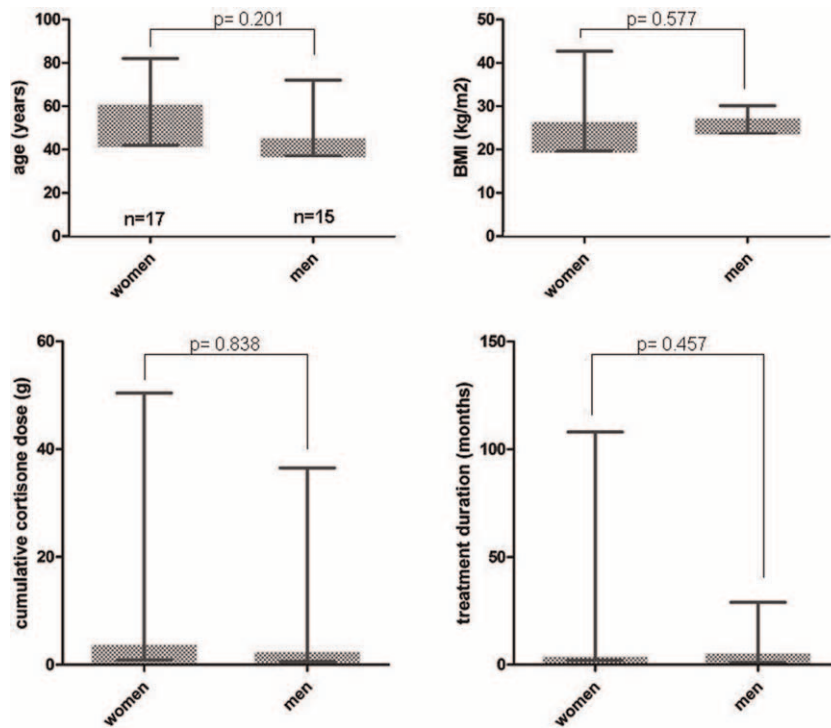
Figure 1. Data (age, BMI, cumulative dose, and treatment duration) are presented as median± range. n=number of patients in each subgroup.

could be the older age (the mean value: 54) of patients with pemphigus and GADA-positive. As the previous authors mentioned, a positive screening test for GADA could be used in patients aged younger than 45 years at diagnosis of DM to indicate those who have an increased risk of requiring insulin therapy.

The effect of steroids on glucose metabolism is the result of multiple pathways including: beta cell dysfunction; decreased
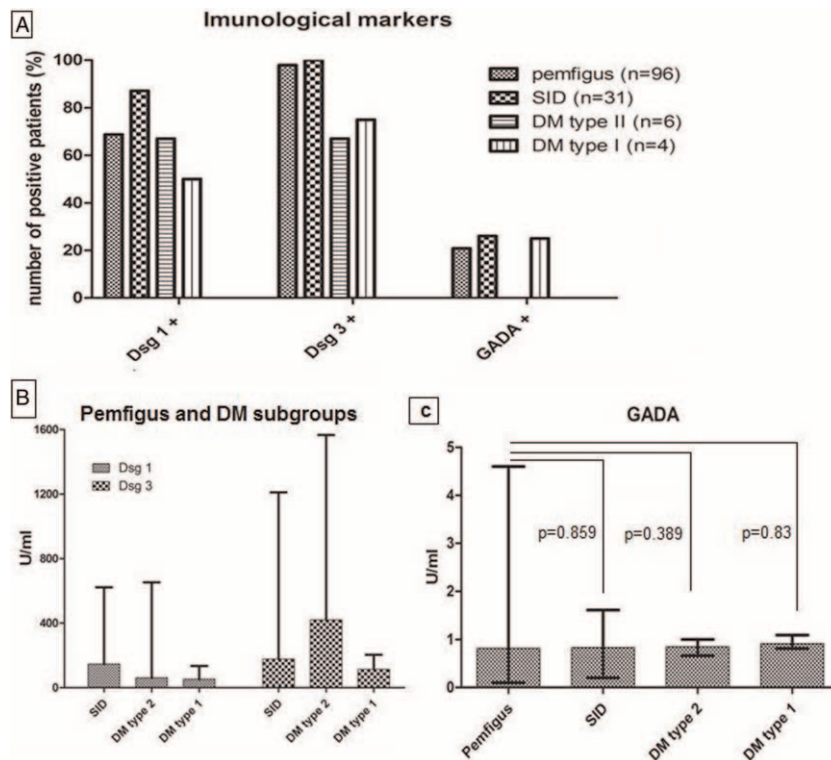

Figure 2. Immunological markers. (A) Number of positive patients; (B) the quantity (unit per milliliter) of desmoglein 1 and; the quantity (unit per milliliter) of GADA.

binding affinity of insulin or decrease receptor number; damage to glyceroneogenesis in liver and adipose tissue; inhibition of post-insulin receptor cascades involving PKB/Akt and mTOR pathways.[11]

There is no consensus regarding the risk factors for SID. Some studies considered as risk factors the cumulative dose, longer duration of steroid course, older age, family history, high BMI, and impaired glucose tolerance.[12] A study conducted by Alavi et al identified corticosteroid-induced hyperglicemia in 40% of patients with pemphigus treated with corticosteroids. In this study, none of the recorded parameters such as age, BMI, family history of DM, corticosteroid dose, and duration of corticosteroid therapy were found to be independently associated with hyperglycemia.[7]

Our study aimed to find other parameters that could relate to the onset of SID, namely immunological markers (desmogleins and GADA). As an autoimmune disease, pemphigus may be associated with other autoimmune disorders and with non-skin-specific autoantibodies, including GADA usually present in type 1 DM.

The simultaneous production of ≥ 2 pathogenic antibodies in patients having a systemic autoimmune disease could be explained by 3 possible scenarios: each T cell epitope, specific for a single disease may associate with its susceptible HLA protein, leading to T cell activation and subsequent stimulation of B cells to produce autoantibodies, leading to dual autoimmunity; each of the disease-specific autoantigens may bind to the HLA protein, inducing both diseases by cross-presentation; and 2 distinct epitopes of the same autoantigen may be able to bind two disease-associated HLA molecules.[13]

From this perspective, we tried a new approach of the patient with pemphigus and SID, but additional studies are needed on a larger number of patients to allow a definitive conclusion.

The prevalence of GADA in the European healthy population was already evaluated using the population from Norway. The authors found a frequency of anti-GADA positivity of 1.7% in a group of 4496 persistently nondiabetic individuals.[14] Anti-GAD positivity in nondiabetic individuals was associated with thyroid-associated autoimmunity and with HLA-DQA1/DQB1 haplotype. In another study, GADA was analyzed in 4976 nondiabetic relatives of type 2 DM patients or control subjects from Finland. A total of 5.9% were GADA-positive.[15] The authors support the idea that the family history for DM increases the risk of type 2 DM. The difference of GADA positivity may be explained by the high rate of type 1 DM in Finland.[16]

In one study published by Mejri et al (2011), GADA were found positive in 1 of 50 (2%) of pemphigus foliaceus patients and none of the patients with pemphigus vulgaris was GADA-positive.[2] Our data show different findings, with a higher incidence of GADA-positive patients in the pemphigus group (21.9%). Possible reasons for the discrepancies with the results obtained by Mejri et al could be because of the heterogeneity of patients groups: differences within the numbers of patients with pemphigus vulgaris (51 vs. 137), sex ratio (female/male: 3:1 vs. 1.5:1), ethnic origin, status of immunosupresion.

Even though the incidence of GADA positive was higher in the patients with pemphigus and SID compared to those without SID, the differences were not statistically significant.

Another hypothesis that should be tested in the following studies would be to determine the ability to secrete insulin in pemphigus vulgaris patients who develop SID. The chronic treatment with glucocorticoids can induce peripheral insulin resistance, resulting in increased endogenous glucose production.

The insulin reserve should be determined before the initiation of cortisone therapy and after the onset of DM, in patients with SID, and comparative in patients without DM, a specific method could be the measurement of C-peptide immunoreactivity[17]

A limitation of our study is related to the insufficient number of subjects and the heterogeneity of our patient groups. One reason for the small number of subjects is the low prevalence of the disease. Also, for a better characterization of our groups, an investigation of the prevalence of anti GADA in healthy population and in a population of type 1 DM patients might be necessary. Further larger studies or meta-analyses including this study are required to further explore this hypothesis.

5. Conclusions

Our study described the incidence of new-onset hyperglycemia in corticosteroid treated-pemphigus subjects, and presented the comparison between pemphigus alone and pemphigus with SID regarding the levels of desmogleins 1 and 3 and GADA. However, the frequency of GADA in patients with pemphigus was high. The risk of developing DM in patients with pemphigus remains a serious problem and is closely associated with the risk of infection and cardiovascular disease, both major causes of mortality in patients with pemphigus.

Author contributions

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Writing – review & editing: Ioana Bâldea, Daniel Leucuta, Gabriel Samasca, Cassian Sitaru, Adrian Baican.

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