

ORIGINAL ARTICLE

Anti-desmoglein 1 antibodies in healthy related and unrelated subjects and patients with pemphigus foliaceus in endemic and non-endemic areas from Tunisia

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Abstract

Background Pemphigus foliaceus is an autoimmune blistering skin disease characterized by the production of pathogenic IgG autoantibodies directed against desmoglein 1.

Aim To determine the prevalence of anti-desmoglein 1 antibodies in healthy subjects and their distribution in the different regions of Tunisia and to better identify endemic areas of pemphigus foliaceus.

Methods We tested, by enzyme-linked immunosorbent assay, sera of 270 normal subjects recruited from different Tunisian areas and 203 related healthy relatives to 90 Tunisian pemphigus foliaceus patients.

Results Seventy-six patients (84.4%), 20 healthy controls (7.4%), and 32 relatives (15.76%) had anti-desmoglein 1 antibodies. In southern regions where pemphigus foliaceus is associated with a significant sex ratio imbalance (9 female : 1 male in the south vs. 2.3 : 1 in the north) and a lower mean age of disease onset (33.5 in the south vs. 45 years in the north), a higher prevalence of anti-desmoglein 1 antibodies in healthy controls was observed (9.23% vs. 5.71% in the north). Interestingly, the highest prevalence of anti-desmoglein 1 antibodies in healthy relatives (up to 22%) was observed in the most rural southern localities. More than half anti-desmoglein 1-positive healthy controls were living in rural conditions with farming as occupation, which suggests that this activity may expose the subjects to particular environmental conditions.

Conclusion These results show that the endemic features of Tunisian pemphigus foliaceus are focused in these southern areas more than in other areas and that both environmental and genetic factors contribute to the disease.

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Keywords

Antibodies, desmoglein 1, endemic, pemphigus foliaceus, Tunisia

Conflicts of interest

None declared.

Introduction

Pemphigus foliaceus (PF) is an autoimmune blistering skin disease mediated by autoantibodies that are specific for the extracellular region of the desmosomal glycoprotein, desmoglein 1 (Dsg1).^{1,2} Anti-Dsg1 antibodies are responsible for the intra-epidermal formation of blisters through the loss of keratinocyte

adhesion, the so-called acantholysis process.³ There are two forms of PF: a non-endemic form, first described in Paris in 1844 that occurs throughout the world, and an endemic form known as fogo selvagem, first reported in Brazil in 1903. Other endemic foci have been reported in Peru,⁴ Colombia⁵ and Tunisia.⁶ Both endemic and non-endemic forms are clinically, histologically and immunologically similar, but several features of the endemic form are unique. For example, in the Tunisian PF, the incidence rate is

Abbreviations: PF, pemphigus foliaceus; Dsg, desmoglein

significantly higher (6.7 cases per million per year) than in France (1.7 cases per million per year), and mainly related to PF cases occurring in young adult women.^{7,8} Indeed, the incidence rate of pemphigus in women aged 25–34 years reaches 20 per million per year, a rate approaching that reported in Brazilian endemic areas. These levels were higher in the rural desert areas of the south.⁷ Furthermore, the discovery of cases among genetically related household members especially in southern Tunisia⁹ is reminiscent of the familial clustering observed in fogo selvagem.

A study performed in Brazil¹⁰ has shown that anti-Dsg1 antibodies can also be detected with a high frequency in healthy household contacts and individuals living in the endemic areas, a serological abnormality that may precede the occurrence of the disease.

Thus, to further characterize the prevalence of anti-Dsg1 antibodies and to discriminate between endemic and non-endemic Tunisian areas, we evaluated the presence of antibodies directed against Dsg1 in patients with PF, in healthy controls from the whole country and in the healthy relatives of the patients. This approach should help (i) to precise the geographical distribution of the biological markers of the auto-immune process and (ii) to better identify endemic areas where we should focus on the search for environmental factors and (iii) the respective role of environment and genetic factors contributing to the disease.

Materials and methods

Subjects

In this case-control study, we recruited 90 PF patients (40 northern patients and 50 southern ones) attending the Dermatology Departments of the University hospitals of Tunis in the north and Sfax in the south. The recruitment started in 1998 in northern regions and in 2002 in southern ones. The diagnosis of PF was established according to the standard clinical, histological and immunological criteria of the disease.¹¹

Two hundred seventy samples from healthy controls originated from the whole country of Tunisia age-matched (± 5 years), gender and location matched (130 from the south and 140 from the north) were included in this study. Healthy controls related to PF patients were excluded. Based on data that identified the features of Tunisian endemic PF, we also recruited 203 related healthy subjects from southern Tunisia. All sera were separated and stored at -80°C until used.

Anti-Dsg1 antibody titration

Anti-Dsg1 antibodies were detected by a commercial ELISA kit (MBL[®], Nagoya, Japan) according to the manufacturer's instructions. The MESACUP DESMOGLEIN 1 TEST is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) used for the detection of IgG anti-Dsg1 antibodies in human sera. Antibody titre was expressed as an index value (U/mL); cut off value was set at 14 U/mL. Titres between cut-off value and 20 were considered as weakly positive.

For IgG subclass studies, the same protocol was applied, but anti-human IgG were replaced by anti-human IgG1, IgG2, IgG3 and IgG4 murine monoclonal antibody (Sigma[®], St. Louis, MO, USA).

Statistical analysis

The statistical program Statistical Purchase for Social Sciences (SPSS) version 13.0 was used for description and inferential statistics. All calculations were carried out with a 95% confidence interval (95% CI). The λ s ratio was calculated according to Rsch's data.¹²

Results

Patients

Tunisian endemic PF has distinct features according to the geographical area: in the southern regions, it is associated with an important sex ratio imbalance (9 female : 1 male in the south vs. 2.3 : 1 in the north) and a lower mean age of disease onset (33.5 years in the south vs. 45 years in the north). Most patients from the south were from rural origin (6 rural : 1 urban), whereas the majority of northern patients were living in urban conditions (1 rural : 2.5 urban).

Forty out of the 50 southern PF sera (80%) exceeded the cut-off value (mean index value, 157 ± 81 U/mL; range, 15–326 U/mL) and 36 of the 40 northern PF patients (90%) had anti-Dsg1 antibodies (mean index value, $221 \text{ U/mL} \pm 86$; range, 49–554 U/mL; Fig. 1). We noted that in the southern group, 9 patients from the 10 in whom antibodies were not detected were in long-term remission.

The distribution of IgG subclasses in Tunisian PF was analysed in 12 southern PF and 9 northern PF sera. Anti-Dsg1 antibodies were of the IgG4 subclass in all cases. IgG2 against Dsg1 was found in 4 southern PF patients (33%) and 6 northern PF (66%), while IgG1 subclass autoantibodies were detected in 2 southern PF patients and 6 northern PF patients. None of the 21 patients had IgG3 anti-Dsg1 antibodies. These findings indicate that IgG4 followed by IgG2 are the dominant IgG subclasses of anti-Dsg1 antibodies in the Tunisian PF.

Controls

Twenty out of the 270 healthy controls (7.4%) had an abnormal anti-Dsg1 antibody titre. Twelve out of 130 (9.23%) among them belonged to the southern population and 8 (5.71%) to the northern population. The index values ranged from 15.4 to 169 (mean value, 38 ± 45 U/mL) and from 18 to 119 U/mL (mean value, 45.5 ± 39 U/mL) for southern and northern controls, respectively (Table 1).

In these 20 anti-Dsg1 Ab-positive healthy control sera, IgG2 against Dsg1 was positive in most cases (14 of 20) and IgG3 anti-Dsg1 antibodies in a few subjects (6 of 20); IgG1 anti-Dsg1 antibodies were rare (1 of 20). All healthy controls were negative for IgG4 anti-Dsg1 antibodies. These data indicate that IgG2 is the dominant IgG subclass of anti-Dsg1 antibodies in the Tunisian healthy controls.

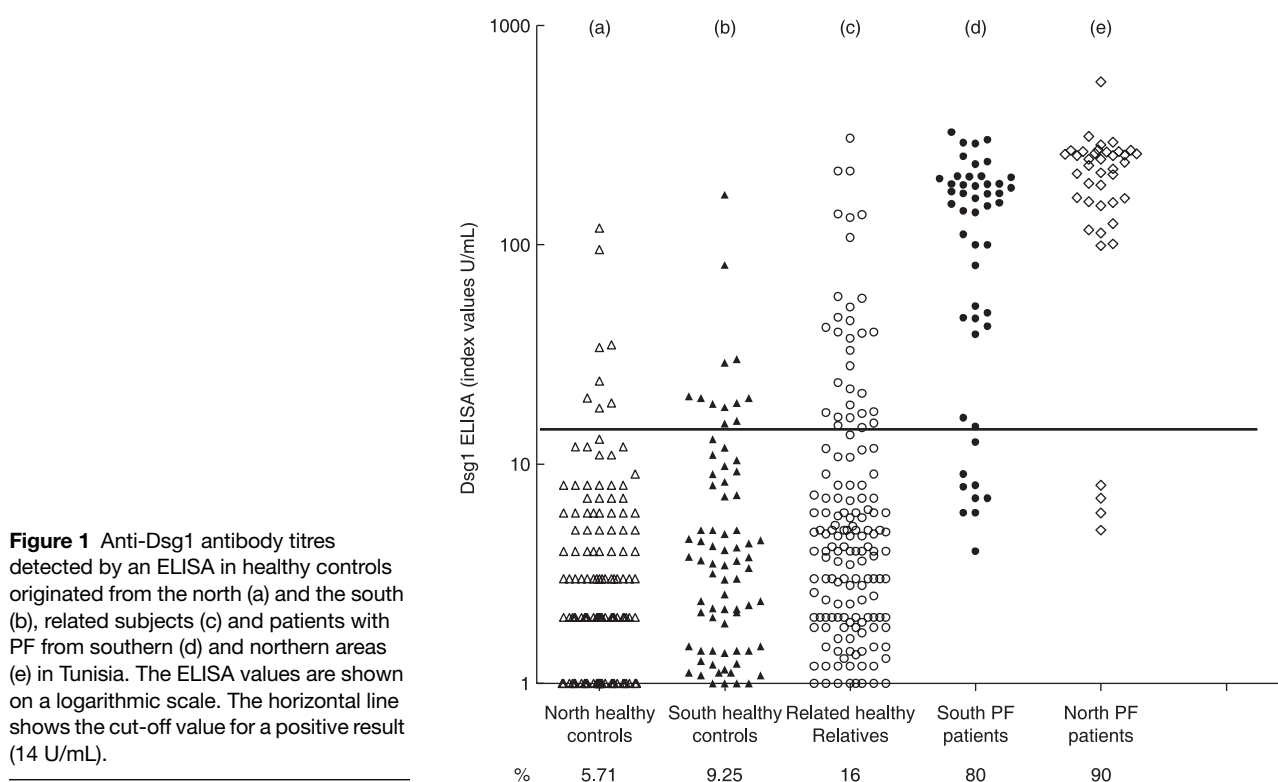


Table 1 Positive tests for antibodies against Dsg1 in patients with PF, healthy controls and related healthy subjects, according to the region in Tunisia

Group and region	Total <i>N</i>	Positive antibody test, <i>n</i> (%)	ELISA value (U/mL)	
			Mean	Range
PF patients				
North	40	36/40 (90)	221	49–554
South	50	40/50 (80)	156	15–326
Healthy controls	270	20 (7.4)	41.75	15.4–169
North	140	8 (5.71)	45.5	18–119
South	130	12 (9.25)	38	15.4–169
Related healthy subjects	203	32 (15.76)	54	15–306
<i>Moknessy</i>				
Unrelated subjects	8	1 (5.55)	169	NA
Related subjects	32	7 (22)	119	17–306
<i>Gabes sub-regions</i>				
Unrelated subjects	21	2 (9.52)	20	NA
Related subjects	83	13 (16)	54	17–217
<i>Rural sub-regions of Sfax</i>				
Unrelated subjects	44	4 (9.09)	18	15–20
Related subjects	53	9 (17)	80	15–217

NA, not applicable.

The demographic characteristics of anti-Dsg1 seropositive healthy controls differed from one region to another. In the south, the Dsg1 seropositivity was associated with an important imbalance in gender (sex ratio F vs. M, 5 : 1 vs. 5 : 3 in the north) and a lower mean age (44.66 ± 13.9 years) than in the north (49.62 ± 21.33 years). Information about living conditions and occupation were available only for 18 of 20 and 11 of 20 healthy controls, respectively. Most of those anti-Dsg1-positive healthy controls were living in rural conditions (12 of 18) with farming as occupation in more than half cases (6 of 11). A significant association was observed between farming and the presence of antibodies against Dsg1 in healthy controls ($P = 0.001$; odds ratio, 7; 95%CI, 1.92–25.81).

Healthy relatives

In areas where Tunisian PF is considered as endemic⁶ (the southern Tunisia), antibodies against Dsg1 were detected in 32 of the 203 healthy related subjects (15.76%). In these regions, there was an increase in the proportion of normal subjects with positive tests for antibodies against Dsg1 ($P = 0.004$) as well as in the mean value of the positive tests in related compared to unrelated healthy subjects (Table 1). Antibody tests were positive in 0 of the 9 subjects from Gafsa (0%), in 1 of the 17 subjects from Kairouan (6%), in 1 of the 9 subjects from Kasserine (11%), in 7 of the 32

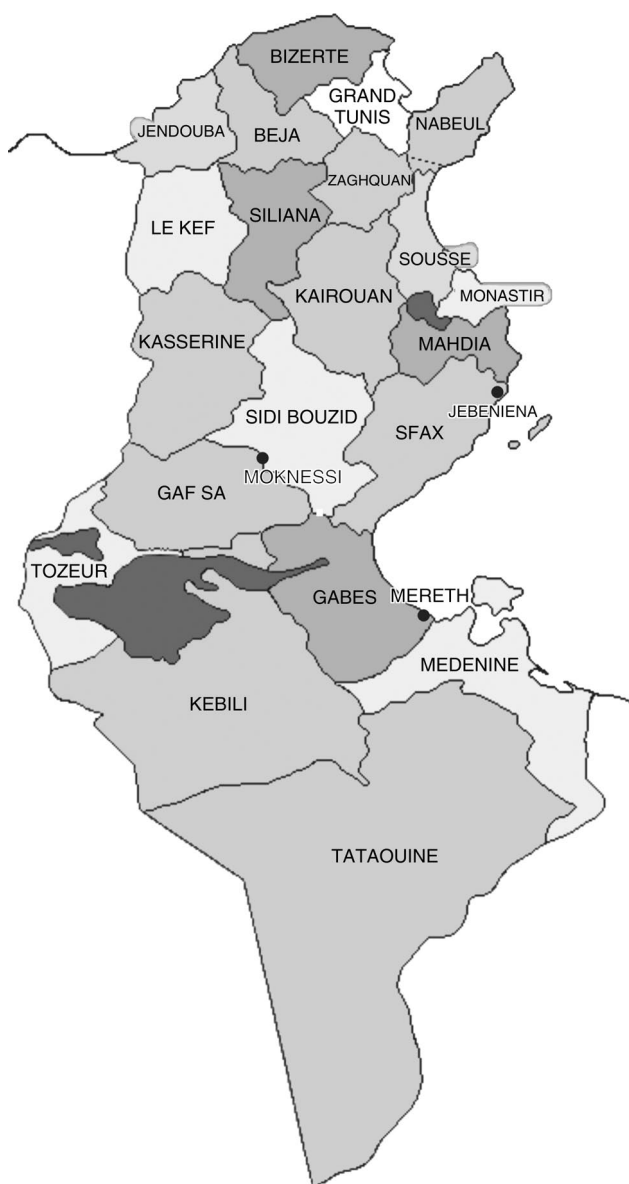


Figure 2 Map of Tunisia showing the different localities and especially the geographical placement of the endemic southern regions: Moknessy, Jebenièna and Mereth.

subjects (22%) from Sidi Bouzid and especially from its sub-region of Moknessy, in 9 from the 53 subjects from the rural sub-regions of Sfax (17%), and in 13 of the 83 subjects from Gabès (16%). We noted that 6 of the 9 anti-Dsg1-positive relatives from Sfax were from the sub-region of Jebenièna, and that 9 of the 13 positive relatives from Gabès were from the locality of Mereth (geographic locations of these regions are shown in Fig. 2). These results indicate that the south of Tunisia, more specifically the rural localities of Moknessy (Sidi Bouzid), Jebenièna (Sfax) and Mereth (Gabès), are those that manifestly express the endemic Tunisian

PF features. Interestingly, in all these sub-regions, the prevalence of anti-Dsg 1 antibodies was higher in healthy relatives of PF patients than in healthy controls recruited in the general population providing a λ s ratio ranging from 1.6 to 4.

Discussion

The demographic characteristics of Tunisian PF in the present series are very similar to those reported in Bastuji's⁸ epidemiological study. Indeed, most cases of our series were young people with a high predominance of women. Our study also emphasizes that pemphigus had distinct features according to the geographical area. In the south of the country, the disease is associated with a significant sex ratio (female : male) imbalance, a lower mean age of disease onset and a higher frequency of subjects from rural origin. These data strengthen the previous observation that the classical features of Tunisian endemic pemphigus are more pronounced in the southern regions compared with the northern ones. This difference in disease characteristics may be the consequence of environmental factors since the southern cohort is composed of patients who still live in their native geographical areas and thus are exposed to the same environment while most northern PF patients were recruited from the capital city Tunis. It may also be due to genetic factors since the ethnic origin of the population differs between the south and the north of the country and the rate of consanguinity is significantly higher in rural southern regions.

As reported in previous studies, anti-Dsg1 antibodies were positive in the majority of patients during the active stage of the disease^{13,14} and IgG4 was the predominant autoantibody subclass.^{15,16} Among the large panel of sera obtained from healthy subjects, 7.4% had antibodies against Dsg1. Even though the difference was not statistically significant, a north-south gradient was observed (5.7% vs. 9%). This prevalence of 7.4% is relatively important compared to that of 0% found in countries where pemphigus occurs as a sporadic form^{17,18} but lower than what is found in Peru (31–46.3%)^{19,20} and in Brazilian regions (39% in the endemic areas of fogo selvagem: Limao verde and 10–28% in its surrounding areas).^{21,22} One should also note that the prevalence of anti-Dsg1-positive healthy controls in Tunisian southern regions where the endemic form occurs is identical to that found in endemic areas of Colombia.¹⁴ In a previous Tunisian study,²³ the prevalence of anti-Dsg1 antibodies reported in healthy controls was 17%. This higher prevalence can be explained by a different mode of healthy control recruitment. Indeed, while in our present study, healthy controls were recruited from all parts of the country and age, gender and location matched to PF patients, in the previous study, they were blood donors recruited from only two towns, Tunis in the north and predominantly Gabès in the south, the latter providing most anti-Dsg1 antibody-positive subjects. In addition, relatives to PF patients were not excluded.

In healthy relatives of PF patients, the frequency of PF autoantibody-positive sera reached 22% in the rural southern

locality of Moknessy (Sidi Bouzid) followed by 17% in Mereth (Gabès) and the rural subregions of Sfax. The observation that the prevalence of anti-Dsg1 antibodies is higher in PF patient relatives than in the general population strongly argues for the participation of genetic factors in the occurrence of Tunisian endemic form of the disease. The additional observation that the majority of PF family cases identified in our country (unpublished data) were also located in these rural southern regions strengthens the role of genetic susceptibility.

Demographic characteristics of anti-Dsg1 antibody-positive southern healthy controls were similar to those of southern PF with predominance in young women aged 30 to 50 years. As previously described in Peru,¹⁹ a significant association was observed between farming and the presence of antibodies against Dsg1, which suggests that this activity may expose them to particular environmental factors present in the endemic areas. In this regard, if the climate conditions in south Tunisia are quite different from those observed in South America endemic regions, the living conditions of the target populations are very similar since they are in close contact with animals and have farming activities.²⁴

Most of anti-Dsg1 antibodies detected in Tunisian southern healthy controls belonged to the IgG2 subclass, while patients had mainly detectable IgG4 followed by IgG2 subclass. This result suggests that progression from the preclinical to the clinical phase of the disease, as observed by Warren *et al.* in Brazilian PF,^{10,24} is closely associated with subclass switching from IgG1 to IgG4 in the Brazilian population and from IgG2 to IgG4 subclass in the Tunisian population.

The high prevalence of PF in our country may also result from inbreeding of population which contributes to the selection of genetically predisposed individuals repeatedly exposed to a sensitizing agent. In this regard, this study raises the issue of susceptibility genes for pemphigus. If we postulate that both patients and normal subjects living in the endemic areas are exposed to the same putative environmental triggering factors, then patients with suitable genetic background are able to make a much stronger pathogenic IgG4 response.

These findings allow us to suggest that exposure to unknown environmental factors is enough for 17–22% of people living in endemic areas to produce a non-pathogenic antibody response. Gene's susceptibility alleles would be required for epitope spreading and class switch leading to the production of pathogenic IgG4 anti-Dsg1 antibodies and the development of the disease.²⁵

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