

Detection of pemphigus autoantibodies in healthy relatives of Turkish patients with pemphigus

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ABSTRACT

Background: Pemphigus autoantibodies have been reported in healthy relatives of pemphigus patients suggesting a genetic predisposition in the pathogenesis of the disease. **Aims:** To test for the presence of pemphigus autoantibodies in healthy relatives of Turkish patients of pemphigus. **Methods:** The study group comprised 45 pemphigus patients, 75 unaffected family members and 47 healthy individuals in the control group. Direct and indirect immunofluorescence techniques were performed to determine the presence of pemphigus autoantibodies. **Results:** By indirect immunofluorescence staining, circulating pemphigus autoantibodies were found in 26.7% of the relatives and in only two of the controls (P value = 0.0001). A direct immunofluorescence technique revealed positive results in three (4%) of the relatives and none of the controls. **Conclusion:** The presence of pemphigus autoantibodies in clinically healthy relatives indicates that genetic predisposition is necessary but not sufficient for the development of clinical disease.

Key words: Family relations, Fluorescent antibody technique, Pemphigus

Pemphigus is a life-threatening autoimmune disease, which is characterized by intraepidermal bulla and acantholysis and is mediated by IgG autoantibodies.^[1] The autoantibodies are thought to be a result of exogenous and endogenous factors.^[2]

Evidences like the frequent occurrence of pemphigus in some ethnic groups such as Ashkenazi Jews and Japanese,^[3] its association with the presence of some HLA alleles,^[4,5] occurrence of familial pemphigus cases^[2,6] and reports of circulating pemphigus autoantibodies in the healthy relatives of pemphigus patients^[1,2,7,8] indicate a role for genetic predisposition in its pathogenesis. We evaluated the frequency of pemphigus autoantibodies in healthy relatives of patients with pemphigus in a Turkish population.

METHODS

Our study included 45 pemphigus patients who were referred to our outpatient clinic between October 1998 and January 2004. The diagnosis of pemphigus was made on the basis of clinical, histological and immunofluorescence examinations. The severity of the clinical disease was assessed to be mild, moderate and severe. For direct immunofluorescence (DIF) examination, a 4 mm punch biopsy specimen was taken from the left arm of the relatives and of the controls. The biopsy specimens were frozen in liquid nitrogen and stored in at -70°C until use. The specimens were cut by cryostat into 5 μ thick sections. After being rinsed with phosphate-buffered saline solution (PBS) for 15 minutes, the slides were incubated with anti-human IgG and complement (Dako-

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polyclonal rabbit) at a dilution of 1/100 in PBS, pH 7.2 at room temperature for 45 minutes. The slides were rinsed three times for ten minutes in PBS.

For indirect immunofluorescence (IIF) tests reactivity of fluorescence intensity was assessed between the titers of 1/10-1/160 for intercellular IgG deposits. For IIF, the blood was drawn from both groups at the time of biopsy. The sera were evaluated by a standard IIF technique using human skin as the substrate.

The analyses were performed using the statistical programme SPSS for Windows 10.0 (Statistical Package for Social Sciences). Chi-square, Fischer's exact test, Mann Whitney U and ANOVA tests were used for the comparison of results. $P < 0.05$ was considered as statistically significant. Prior approval of the local ethics committee was taken and informed consent was obtained from participants.

RESULTS

Thirty-two of the patients were women (71.1%) and 13 were men (28.9%). Their mean age was 52.3 years (range = 24-74 years). The pemphigus subtypes were as follows: four patients with pemphigus erythematosus (8.9%), one patient with pemphigus foliaceus (2.2%), one patient with pemphigus herpetiformis (2.2%) and 39 patients with pemphigus vulgaris (86.7%). The disease activity was mild in four (8.9%), moderate in 35 (77.8%) and severe in six (13.3%) patients.

The study also included 75 first-degree healthy relatives of which 32 were women (42.7%) and 43 were men (57.3%) with a mean age of 29.6 years (range = 7-77 years). A control group consisting of 47 healthy individuals, of which 28 were women (59.6%) and 19 were men (40.4%), the mean age being 36.4 years (range = 12-74 years), was also included.

DIF revealed negative results in 72 (96%) of the relatives while it was positive in three (4%). IIF was also positive for two of these three relatives. There were no positive DIF results in

the control group. IIF was positive in 20 (26.7%) relatives. Of the 47 individuals in the control group, 45 (95.7%) showed negative IIF results while two (4.3%) showed positive results. In six (13.3%) of the pemphigus patients, IIF gave negative results [Table 1].

There were no statistically significant differences in the frequency of positive DIF results between the relatives of the pemphigus patients and the control group. The frequency of IIF positivity in the relatives' group was found to be significantly high when compared to that of the control group (26.7% vs 4.3%) ($P < 0.001$). No statistically significant correlation was found between clinical severity of the disease in the patients' group and the frequency of IIF positivity in the relatives' group ($P > 0.05$).

DISCUSSION

Pemphigus vulgaris (PV) is strongly associated with some major histocompatibility (MHC) alleles. The alleles that are frequently found are DR4 and DQ8 in Jewish patients and DR6 and DQ5 in non-Jewish patients.^[9] The relationship between pemphigus and MHC has been well demonstrated but only a few cases of familial pemphigus have been reported. Beutner and Chorzelski^[10] found only one case of familial PV in their series of 234 cases. Reohr *et al.*^[11] studied the MHC of two siblings with PV by restriction fragment polymorphism methods and found that both of them had DR4 and DQW3.2 alleles. Though their father had the same haplotypes, he did not develop clinical signs of PV.

In many studies, low titers of pemphigus autoantibodies were found in the first-degree relatives of pemphigus patients. Ahmed *et al* found low levels of PV autoantibodies in 48% of 120 healthy first-degree relatives of 31 PV patients and they linked the presence of these autoantibodies to HLA-DR4 or DR6 haplotypes of the patients.^[9] Kricheli *et al.* detected pemphigus autoantibodies in 15% of 55 healthy relatives of 25 pemphigus patients by using the IIF technique. However, none of the 56 controls had PV-IgG in their sera.^[8] The antibody

Table 1: The results of direct immunofluorescence and indirect immunofluorescence techniques in pemphigus patients, relatives and control group

	Relatives		Control group		Pemphigus patients		Chi square	P value
	n = 75	%	n = 47	%	n = 45	%		
Direct immunofluorescence								
Negative	72	96.0	47	100.0	0	0		
Positive	3	4.0	0	0.0	45.0	100.0		0.284
Indirect immunofluorescence								
Negative	55	73.3	45	95.7	6	13.3		
Positive	20	26.7	2	4.3	39	86.7	73.05	0.0001***

*** $P < 0.001$ statistically very significant

titers of the relatives ranged from 1/10 to 1/160. Brandsen *et al* searched for the presence of pemphigus autoantibodies by DIF, IIF and immunoblotting techniques in 21 first-degree relatives of 12 pemphigus patients and a control group consisting of 30 individuals.^[2] They found a higher incidence of PV-IgG (71%) by using the three techniques. These IgGs were demonstrated to bind the epidermal cells in only five (25%) of the relatives. There was no positivity found in the control group by any of the methods used.

In a series of 12 Italian pemphigus patients, a significant number of the 67 first-degree relatives had PV-IgG as determined by IIF (31%) and immunoblotting methods (47%).^[7] The antibody titers were between 1/20 and 1/80. In another survey of 56 first-degree relatives of 37 pemphigus cases, the incidences of pemphigus autoantibodies were 42.8 and 19.8% by IIF and ELISA methods, respectively.^[1] In the present study, we found pemphigus autoantibodies in 26.7% of the relatives. These results were between 15-31.1%, a range that has been detected in previous studies.^[7,8] The range of antibody titers (1/10 to 1/160) detected in our study, was consistent with the range reported in Kricheli's study.^[8] On the other hand, the rate of positivity detected by DIF (4%) in our study was significantly lower than the rate that Brandsen *et al* determined (25%). This situation may be due to the small sample size that they had used.^[2]

Although there is a genetic predisposition in pemphigus, the question as to why the relatives of the patients do not show clinical signs of the disease remains unanswered. Kricheli *et al* suggest that the antibody titers detected in the sera of the relatives were not high enough to bind the pemphigus antigens in the skin and induce acantholysis. Thus, the disease may be triggered by infections or drugs.^[8] These authors also found that the distribution of PV IgG1-3 subclasses were similar among patients and their relatives but PV IgG4 was higher in patients and lower in relatives. This finding may indicate the role of PV IgG subclasses in the pathogenesis of the disease.

In almost all of these studies, circulating antibodies have been well detected by the IIF technique while the DIF results were usually negative. This suggests an immunologic or mechanical barrier that interferes with the interaction between circulating IgG and the skin. This barrier may be demolished by drugs, malignancies, viral infections or burns, which may result in clinical disease. The variability of the

susceptibility of keratinocytes or the penetrability of the epidermis may thus determine who develops clinical disease and who remains asymptomatic with circulating pemphigus antibodies.^[2] On the basis of the previous and present studies, we conclude that though immunogenetic factors constitute a basic part in the pathogenesis of pemphigus, they are not sufficient for the development of the disease. Details of interactions between genetic and environmental factors need further illumination.

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