

ELECTROPHORETIC AND IMMUNOELECTROPHORETIC STUDY OF THE SOUTH AMERICAN PEMPHIGUS FOLIACEUS

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SUMMARY

Sera from 21 patients of South American Pemphigus Foliaceus were studied by electrophoretic and immunoelectrophoretic techniques.

Increased values of alpha₂-globulin were observed in 66.6 per cent of the patients. No simultaneous infections could be detected suggesting that this increase was related to the Pemphigus itself.

Hypergammaglobulinemia was the most frequent finding.

IgA was increased in 28.6 per cent of the cases and IgM was absent in 23.8 per cent.

Considering the total number of cases in which at least one of the immunoglobulins was deficient it was noted that 43.0 per cent of the patients had immunoglobulin deficiency; this establishes correlations between this pathology and immunological mechanisms.

INTRODUCTION

South American pemphigus foliaceus (fogo selvagem — wildfire pemphigus) has not been well explored from the etiopathogenic point of view. Clinically and histopathologically, South American pemphigus foliaceus does not differ fundamentally from the Case-nave's disease described in 1850. However, there are distinctive epidemiological features in Brazil and neighbouring countries.

The results of BEUTNER et al.¹ and LEME⁸, suggested an autoimmune mechanism in the pathogeny of the South American pemphigus foliaceus. It was thought therefore of interest to perform a detailed analysis of the serum protein fractions including immunoglobulins

of the blood of patients with this disease. The data on of this subject are scarce.

We present here the data that show distinct changes in the electrophoretic and immunoelectrophoretic pattern of the serum from patients with South American pemphigus foliaceus.

Only the results of FURTADO & RODRIGUES⁴, who showed paper electrophoresis in the sera from patients of South American pemphigus foliaceus and MARTINA⁹ that titrated the IgA, IgG and IgM by immunoplate technique in sera from patients of pemphigus vulgaris could be found in the literatura available.

This research was supported in part by a grant from "Fundação de Amparo à Pesquisa do Estado de São Paulo"

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MATERIAL AND METHODS

Serum samples from 21 patients (11 females and 10 males) of South American pemphigus foliaceus were collected at the moment of admission to the Pemphigus Foliaceous Hospital of São Paulo. The age of these patients were between 5 and 65 with a mean age of 37 years.

Cases were classified (Table I) according to the severity of the disease into:

- Grade 1 — Benign form (Pemphigus erythematosis, Senear-Usher disease).
- Grade 2 — Disseminated form, present in subjects with up to 40 years of age, not associated to other severe diseases or toxemia, but affecting about 50 per cent of the body surface.
- Grade 3 — Patients with disseminated form associated to another severe disease. Toxemia was present in these cases and over 50 per cent of the body surface was involved. Patients who did not respond to corticoid therapy were also included in this group.

TABLE I

Grade	Number of patients
1	3
2	16
3	2

Regarding the treatment with corticosteroids, 7 patients had not been treated while 14 had this compound before entering the hospital (see Table II). At the moment of blood collection, all of them presented clinical evidences of active disease.

As South American pemphigus is prevalent mostly in rural areas, all of the patients were investigated for other diseases which could influence the protein distribution, such as Chagas disease, Manson's schistosomiasis and South American Blastomycosis.

Three out of the 21 patients showed a positive complement fixation test for American Trypanosomiasis (patients numbers 6452, 6536 and 6626), although none of them, in the whole group, exhibited clinical manifestations of Chagas disease.

Complement fixation and precipitation tests for South American Blastomycosis were also performed and resulted negative in all patients. Patient number 6491 had diabetes, and had never been submitted to therapy. All the patients did not present Manson's schistosomiasis.

METHODS

- 1 — Total protein was determined by the biuret reaction according to GORNALL et al.⁵
- 2 — Paper electrophoresis was made according to the technique of GRASSMANN & HANNIG⁷, with some minor modifications.
- 3 — The immunoelectrophoresis of GRABAR & WILLIAMS⁶ was used with modifications as described by FERRI & COSSERMELLI².

RESULTS

The results obtained are presented in Tables II and III.

1 — TOTAL PROTEINEMIA

The mean value of total protein in the 21 samples was 7.0 g/100 ml, but 4 patients presented total proteinemia below 6.0 g/100 ml and 3 above 8.0 g/100 ml.

2 — PAPER ELECTROPHORESIS

2.1 — The mean albuminemia was low either considering the relative (per cent) or the absolute (g/100 ml), values. Only five patients presented albuminemia within the normal range. The others had hypoalbuminemia,

TABLE II
Electrophoretic, immunoelectrophoretic and other data related to the patients of South

	Paper Electrophoresis											Immuno-electrophoresis		
	Relative concentrations					g/100 ml								
Patient number	Albumin	α_1	α_2	β	γ	T.P.	A	α_1	α_2	β	γ	IgA	IgM	IgG
6621	48.7	6.1	9.6	12.2	23.4	7.9	3.86	0.48	0.76	0.96	1.85	N	↑	↑
6619	55.3	5.6	8.8	11.6	18.2	6.8	3.79	0.38	0.60	0.79	1.24	N	N	N
6620	43.5	6.4	12.4	14.5	23.2	8.1	3.53	0.52	1.00	1.17	1.88	N	N	↑
6452	25.5	10.7	13.6	14.4	35.8	6.0	1.54	0.64	0.81	0.86	2.15	N	N	↑
6475	29.4	6.7	9.2	9.2	45.4	6.4	1.88	0.43	0.59	0.59	2.91	N	↓	↑
6491	28.4	6.2	23.3	14.7	27.4	5.7	1.62	0.35	1.33	0.84	1.56	↑	A	N
6502	31.3	11.9	15.9	12.7	28.2	5.1	1.60	0.60	0.81	0.65	1.44	↑	↓↓	N
6522	50.3	4.3	8.2	12.1	25.1	6.9	3.47	0.30	0.56	0.83	1.74	↑	↑	N
6536	47.1	6.4	7.7	11.6	27.2	7.6	3.58	0.49	0.58	0.88	2.07	N	N	↑
6535	40.4	7.3	11.8	10.1	30.4	7.9	3.20	0.57	0.93	0.80	2.40	N	↑	↑
6532	37.0	6.2	15.9	13.6	27.3	7.3	2.70	0.45	1.16	0.99	2.00	N	N	↑
6531	42.6	7.2	11.2	17.0	22.0	6.6	2.81	0.47	0.74	1.12	1.46	N	N	N
6545	32.0	11.1	14.5	15.8	26.6	5.2	1.66	0.58	0.75	0.82	1.39	A	↑	N
6550	43.0	7.6	11.4	10.7	27.3	7.3	3.14	0.55	0.83	0.78	2.00	↑	N	↑
6565	39.3	9.9	11.7	9.9	29.2	6.7	2.63	0.66	0.78	0.66	1.97	N	↓	↑
6573	34.4	6.0	13.4	15.1	31.1	7.3	2.51	0.44	0.98	1.10	2.27	↑	N	↑
6578	37.4	10.0	13.4	14.6	24.6	5.4	2.02	0.54	0.72	0.79	1.33	N	A	N
6584	38.4	8.8	14.9	13.5	24.4	7.3	2.80	0.64	1.10	0.98	1.78	N	A	N
6527	34.3	4.4	9.3	16.2	35.8	7.7	2.64	0.34	0.72	1.25	2.75	N	A	↑
6626	58.0	2.7	8.7	9.8	20.8	9.3	5.40	0.25	0.81	0.91	1.93	↑	↑	↑
6625	39.9	5.3	12.0	12.0	30.8	8.3	3.31	0.44	1.00	1.00	2.55	N	A	↑
Arithmetic means:	39.8	7.2	12.2	13.0	27.8	7.0	2.84	0.48	0.84	0.90	1.94			

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American pemphigus foliaceus

Grade	Previous treatment		Lasting of sickness	Age (Years)	Observations
	Corticoid	Time of application			
2	P	0	7 years	30	
2	T	4 months		53	
1	T	6 months		55	
2	T	2 months		35	* Machado-Guerreiro (+)
2	T	1 year	9 years	25	
3	None			65	Diabetes
2	None			17	
1	None		6 months	57	
1	None			36	Machado-Guerreiro (+)
2	Several			17	
2	None			5	
2	Several		1 year	59	
2	B			55	
2	Several	30 days	1 year	18	
2	T		5 years	24	
2	None			39	
3	T	2 months	6 years	44	
2	None		3 months	14	
2	P			58	
2	P			27	Machado-Guerreiro (+)
2	P	15 days		45	

P = Prednisone or Prednisolone

= Triancinolone

B = Betamethasone

* = Reaction for American trypanosomiasis

TABLE III
South American pemphigus foliaceus

21 patients	IgA	IgM	IgG
Increased	28.6	23.8	62.0
Decreased	—	14.3	—
Normal	66.6	38.1	38.0
Absent	4.8	23.8	—

the lowest being 28.4 per cent of the total protein or 1.62 g/100 ml (Table II).

2.2 — The levels of alpha₁-globulin were normal, but 4 patients (the same ones with hypoprotei-
nemia) exhibited an increase of this globulin considering the relative values.

2.3 — Increased values of alpha₂-globulins were observed in 66.6 per cent of the patients which had more than 12.0 per cent of the total protein. All the patients with increased alpha₁, presented also increased alpha₂, but there were some cases in which the alpha₂-globulin was increased and the level of alpha₁ was normal.

2.4 — In spite of being under steroid treatment, several patients had normal levels of beta globulin with only 3 exceptions (sera number 6531, 6545 and 6527) with slightly increased values.

2.5 — Hypergammaglobulinemia was the most frequent alteration; even in the cases in which the absolute values were not increased, percentual distribution of this fraction was higher in the electrophoretic gammaglobulin.

However, the increase of this globulin reached very high levels only in a few cases in which more than 30 per cent of the total protein were represented by this protein fraction.

3 — IMMUNOELECTROPHORESIS

IgA — In spite of the difficulty of evaluating this immunoglobulin by immunoelectrophoresis, it was observed that in 66.6 per cent of the cases (Table III) the intensity of its precipitin line was normal, whereas increased intensity was seen in 28.6 per cent of the cases, while this immunoglobulin was absent in 4.8 per cent of the patients. Anyway, it is necessary to point out that it is difficult to evaluate IgA because its precipitin line is sometimes superposed to the IgG line.

IgG — This class of antibodies was increased in 62.0 per cent of the patients and within normal range in 38.0 per cent.

IgM — The results of the immunoelectrophoresis have pointed out the very important role of the 19 S antibodies in South American pemphigus foliaceus, since 23.8 per cent of the patients lack this immunoglobulin. Only one of such patients lacking IgM was under treatment with steroids. If one considers the whole lot of patients with absence or at least deficient level of one of the immunoglobulins, an immediate conclusion arises: some immunological deficiency might be related to this pathology, since 42.9 per cent of the cases presented it.

COMMENTS

Normal values for total protein and electrophoretic fractions obtained by FERRI et al.³ were taken as control for the normal range.

Total proteinemia in South American pemphigus foliaceus was within normal range, as previously described by FURTADO & RODRIGUES⁴.

Although the mean values for alpha₁ and beta globulins were also within the normal range and alpha₂ slightly above (12.2 per cent), when individual values were considered, it was noted that 66.6 per cent of the patients presented increased values for alpha₂ globulin. In these cases no simultaneous secondary infections could be detected suggesting that the increase of alpha₂ in these patients was related to the pemphigus foliaceus itself. In this laboratory, the upper normal value for alpha₂ was found to be 11.16 (FERRI et al.³).

In some patients very high levels of alpha₂-globulin (i.e. 23.2 per cent of the total protein) were found. This figure is not in agreement with the values found by FURTADO & RODRIGUES⁴.

The most frequent and important electrophoretic finding was hypergammaglobulinemia, although some of the patients presented normal levels. It must be emphasized, however, that most of the patients had been treated with steroids before blood sampling.

The immunoelectrophoretic study of the immunoglobulins showed some alterations that must be considered, since an autoimmune mechanism is probably related to the pathogeny of South American pemphigus foliaceus^{1, 3}: increase IgA was observed in 28.6 per cent of the patients, in agreement with Martina's⁹ observation in pemphigus vulgaris; IgM globulin was absent in 23.8 per cent of the patients. Martina found values equivalent to half of the normal level of IgM in pemphigus vulgaris.

Absence of IgM could not be linked to any especial infectious process, that could be responsible for its consumption.

Considering the total number of cases in which at least one of the immunoglobulins was decreased or absent, we found that 43.0 per cent of the patients presented an immunological deficiency in the circulating immunoglobulins.

It is very difficult to conclude whether these immunoglobulin deficiencies, especially IgM, are due to an increased consumption, or to a primary deficiency of immunoglobulin producing cells.

Two out of the five patients lacking IgM had a positive reaction for American trypanosomiasis, which shows that this immunological deficiency is not related to this infection, since this disease promote a significant increase of IgM*.

From the study of our data, no correlation could be found between clinical grades, steroid therapy, duration of the disease or presence of secondary infections and decreased levels of immunoglobulins.

RESUMO

Estudos eletroforético e imunoeletroforético do pênfigo foliáceo sul-americano

Foram estudados por análise eletroforética e imunoeletroforética, soros de 21 pacientes de pênfigo foliáceo sul-americano (fogo selvagem).

Observou-se que em 66,6% dos pacientes a alfa₂-globulina estava aumentada sendo êste desvio atribuído ao pênfigo e não a infecções secundárias. Hipergamaglobulinemia foi a alteração mais freqüente.

IgA estava aumentada em 28,6% dos casos, IgM ausente em 23,8% e considerando-se o número total de casos em que houve deficiência de pelo menos uma das imunoglobulinas obtém-se o percentual 43,0, o que estabelece certa correlação entre esta patologia e mecanismos imunológicos.

Estudo evolutivo dos mesmos pacientes encontra-se em andamento.

ACKNOWLEDGMENTS

We are indebted to Dr. J. A. Campos, Director of the Pemphigus Hospital in São Paulo for the facilities in the obtention of the material, to Prof. Celeste Fava Netto for the complement fixation tests for South American Blastomycosis and to Mr. Gilberto Siqueira Lopes for the Machado-Guerreiro tests for American Trypanosomiasis.

* Observation carried out in this Laboratory

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Recebido para publicação em 15/5/1970.