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# Antiphospholipid antibodies in leprotic patients: A correlation with disease manifestations

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**Key words:** aPL, leprosy, aCL, 2-GPI, antiprothrombin.

# ABSTRACT Objectives

Previous studies showed that antiphospholipid antibodies (aPL) are frequent in the sera of leprosy patients and are most probably directed against body tissue cardiolipins. Some groups have demonstrated differences between the binding specificity of "autoimmune-aPL" and "non-autoimmune-aPL". It is widely accepted that a plasma protein,  $\beta$ 2-Glycoprotein I ( $\beta$ 2-GPI), is required for the binding of autoimmune anticardiolipin antibodies (aCL) to cardiolipin. However, some reports suggested heterogeneity of leprosy aCL with respect to their B2-Glycoprotein I (B2GPI) dependency, although no thromboembolic complications have been reported. This study was designed to assess the specificity of aPL by investigating the prevalence of aCL, anti-phosphatidylserine (aPS), anti-phosphatidylinositol (aPI), antiβ2GPI and antiprothrombin (aPT) antibodies, and evaluate their clinical significance in a group of patients with lepromatous leprosy.

# **Patients and methods**

35 lepromatous leprosy patients were selected randomly from an Egyptian leprosarium as a study group. 35 normal household contact controls were selected matching the study group for both sex and age. aCL, aPS, aPI, aPT, anti- $\beta$ 2GPI and  $\beta$ 2-dependent aCL antibodies were investigated by ELISA in all patients and controls.

# Results

aCL antibodies were more frequent in leprosy patients than in controls [13/35 (37%) vs. 3/35 (9%), respectively, p =0.02] and significantly correlated with Raynaud's phenomenon, skin nodules, chronic skin ulcers and urticarial skin rash. No association was found with hypopigmentation, hyperpigmentation and saddle nose. None of the patients presented aPS nor aPI. Only 1 subject from the control group presented aPI along with aCL. aPT were present in 2/35 (5.7%) and anti- $\beta$ 2GPI in 1/35 (2.9%) leprotic patients. None of the individuals from the control group presented aPT nor anti-\beta2GPI.

# Conclusions

An association was found between the presence of aCL and certain dermato-

logical manifestations of leprosy, such as Raynaud's phenomenon, skin nodules, chronic skin ulcers and urticarial skin rash. As in other infectious diseases, there was a lack of  $\beta$ 2GPI-dependency and an absence of thrombotic complications.

#### Introduction

Antiphospholipid antibodies (aPL) represent a large group of immunoglobulins of considerable clinical importance due to their association with arterial and/or venous thrombosis and pregnancy morbidity, condition termed antiphospholipid syndrome (APS) (1). The APS is now recognized as the most common cause of acquired thrombophilia (2). In clinical practice, both anticardiolipin antibodies (aCL) detected by ELISA and lupus anticoagulant (LA) detected by clotting assays have been the most established and standardized tests for the diagnosis of the APS.

The presence of aPL have been reported not only in autoimmune diseases but in a variety of other diseases including infections (e.g. syphilis, AIDS), malignancies and exposure to certain drugs (e.g. phenothiazines and hydralazines), conditions not associated with thromboembolic complications (3). Although with low prevalence, aPL have been found in normal population (4). Some groups have demonstrated differences between the binding specificity of "autoimmuneaPL" and "non-autoimmune-aPL". It is widely accepted that a plasma protein,

2-Glycoprotein I (2-GPI), is required for the binding of autoimmune aCL to cardiolipin (CL) (5, 6).

Leprosy is a mycobacterial disease, primarily affecting the peripheral nervous system, and secondarily involving skin and certain other tissues. It presents a broad spectrum of clinical lesions (7) and a high frequency of autoantibodies, especially aCL (8, 9). aCL antibodies are present in both multibacillary (MB) and paucibacillary (PB) leprotic patients' sera being more prevalent in the MB type (10). Despite the chronicity of leprosy and the persistence of aCL (11-14), no aPL-related thromboembolic complications have been reported.

Antibodies detected in the aCL assay are heterogeneous, and include both antibod-

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ies to CL and antibodies to CL-bound plasma proteins (e.g. 2-GPI). In leprosy it is suggested that aCL are most probably directed against body tissue CL since corynaebacteria leprae does not contain CL nor phosphatidylethanolamine and the proportions of phosphatidylinositol and phosphatidylglycerol are very low (13). Some authors have reported that, in leprosy, aCL are heterogeneous with respect to their requirement for binding to CL, showing that some of these aPL could also be of the 2-GPIdependent type (12, 15).

This study was designed to assess the specificity of aPL by investigating the prevalence of aCL, anti-phosphatidyl-serine (aPS), anti-phosphatidylinositol (aPI), anti- 2GPI and anti prothrombin (aPT) antibodies, and evaluate their clinical significance in a group of patients with lepromatous leprosy.

# Patients and methods

# Patients

This study comprised 35 lepromatous leprosy patients, selected randomly from an Egyptian leprosarium (12 females; mean age 43.8  $\pm$  17.7 years). The duration of the disease was estimated in 15.2  $\pm$  9.2 years. Thirty-five normal household contacts were allocated as control group, matching the study group for both sex and age (15 females; mean age 40.6  $\pm$  14.8 years).

# Methods

The aCL ELISA was performed according to the standardized technique (16). Anti- 2GPI antibodies, using human purified 2GPI and irradiated ELISA plates, were tested as previously described (17). 2-GPI dependent aCL were detected by ELISA as previously described (18). aPT were tested by ELISA using purified human prothrombin as previously described (19).

Multiple aPL ELISA was performed as previously described (20). Briefly, microtiter ELISA plates (Immulon 1, Dynatech Inc., Virginia, USA) were coated with 50  $\mu$ g/ml of phosphatidylserine (PS) and phosphatidylinositol (PI) (all from Sigma, USA), in chloroform:methanol (1:4) solution and dried. After blocking with 10% adult bovine serum (Sigma) in phosphate buffered saline Table I. Frequency of aCL, aPS, aPI, aPT and anti- 2GPI in leprotic patients and controls.

|                           | aCL<br>n (%) | aPS<br>n (%) | aPI<br>n (%) | aPT<br>n (%) | anti- 2GPI<br>n (%) |
|---------------------------|--------------|--------------|--------------|--------------|---------------------|
| Leprosy patients (n = 35) | 13 (37)      | 0            | 0            | 2 (5.7)      | 1 (2.9)             |
| Controls $(n = 35)$       | 3 (9)        | 0            | 1 (2.9)      | 0            | 0                   |
| Z test                    | 3.08         | -            | 0            | 0            | 0                   |
| p                         | 0.02         | -            | 1            | 1            | 1                   |

(10% ABS-PBS), serum diluted 1:100 in 10% adult bovine serum (10% ABS) was added in duplicate. After incubation for 3 hours and 3 washes with PBS, alkaline phosphatase conjugated goat antihuman IgG or IgM was added in the appropriate dilution. Colour was developed by adding 100  $\mu$ l of 1mg/ml of p-nitrophenylphosphate disodium in 1M diethanolamine buffer (pH 9.8). Plates were incubated until the optical density (OD) of a high binding serum (positive control) reached 1.0. Results were expressed as a Binding Index calculated as follows:

BI = [OD (sample) - OD (blank)]

[OD (positive control) - OD (blank)] Values greater than 5 standard deviations above the mean of healthy controls were considered positive.

#### Statistical analysis

A two-tailed t-test for paired data was used to compare sample means and the z test (Poisson rates) was used to compare the percentages (rates) of samples. Correlation was done using two tests: the Sign test for qualitative correlation (correlation of incidence of related samples regardless of their levels), and Spearman's rank correlation coefficient for quantitative correlation (correlation of incidence and levels of related samples). Quantitative correlation is not considered in the absence of qualitative correlation, while qualitative correlation is considered even in the absence of quantitative correlation.

# Results

#### Prevalence of aPL

aCL antibodies were more frequent in leprosy patients than in controls [13/35 (37%) vs. 3/35 (9%), respectively, p =0.02]. None of the patients presented aPS nor aPI. Only 1 subject from the control group presented aPI along with aCL. aPT were present in 2/35 (5.7%) and anti 2-GPI in 1/35 (2.9%) leprotic patients. None of the individuals from the control group presented aPT nor anti- 2GPI antibodies. The frequency of aCL, aPS, aPI, aPT and anti- 2GPI in leprotic patients and controls is shown in Table I.

# aPL and clinical manifestations of leprosy

The presence of aCL was associated with some dermatological lesions in leprotic patients. Positive aCL antibody test correlated qualitatively with Raynaud's phenomenon, chronic skin ulcers, urticarial skin rash and skin nodules. No correlation was found with hypopigmentation, hyperpigmentation and saddle nose (Table II). There was no significant quantitative correlation between IgG or IgM aCL with leprotic manifestations. No association was found between the presence of aPS, aPI, aPT or anti 2-GPI and clinical manifestations of leprosy.

# Discussion

Leprosy is a mycobacterial disease in which a high frequency of autoantibodies, especially aCL, has been reported (8, 9). Despite the chronicity of leprosy

**Table II.** Association between the presence of aCL and clinical manifestations of leprosy.

|                     | Leprosy patients $n = 35$ | aCL<br>positive<br>n = 13 | Sign<br>test |
|---------------------|---------------------------|---------------------------|--------------|
| Raynaud's phen.     | 11                        | 6                         | 0.005        |
| Chronic skin ulcers | 6                         | 4                         | 0.001        |
| Urticarial rash     | 6                         | 5                         | 0.0001       |
| Hypopigmentation    | 16                        | 6                         | 0.45         |
| Hyperpigmentation   | 9                         | 2                         | 0.45         |
| Skin nodules        | 7                         | 4                         | 0.005        |
| Hair loss           | 10                        | 4                         | 0.15         |
| Eyebrow loss        | 20                        | 5                         | 0.99         |
| Saddle nose         | 16                        | 6                         | 0.45         |
|                     |                           |                           |              |

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and the persistence of aCL (11-14), no aPL-related thromboembolic complications have been reported.

The prevalence of aCL in leprosy has been previously studied. Furukawa et al. found increased aCL levels in 20% of patients with leprosy (21). Hojnik et al. detected aCL antibodies in 98% of the 61 patients with lepromatous leprosy (12). Interestingly, they reported that the frequency of these antibodies was decreased to 52% in the absence of 2-GPI, suggesting heterogeneity of leprosy aCL with respect to their 2-GPI requirement for binding to CL. Moreover, in a recent study, Fiallo et al. (15) suggested that aCL in MB leprosy patients are mainly of the 2-GPI-dependent type and emphasize the importance of 2-GPI addition for aCL titration in leprosy.

We found a prevalence of aCL of 37% in patients with lepromatous leprosy. None of our patients presented 2-GPI-dependent aCL.

High levels of anti- 2-GPI antibodies have been reported in 18% of leprotic patients (12). In our series, only 1 patient was positive for anti- 2GPI and none for aPT, suggesting that these antibodies might be considered as an expression of the broad autoimmune syndrome involving the phospholipid binding plasma proteins rather than polyclonal B cell activation.

From our data, we can conclude that an association was found between the presence of aCL and certain manifestations of leprosy, such as Raynaud's phenomenon, chronic skin ulcers, urticarial skin rash and skin nodules. As in other infectious diseases, although a high prevalence of aCL was found, there was a lack of 2-GPI dependency and an absence of thrombotic complications at least in this group of patients.

We believe that the positive association of aPL with disease manifestations of leprosy deserves further study.

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