# Anticardiolipin and antinuclear antibodies in the adult healthy Omani individuals

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## ABSTRACT

**Objective:** To investigate the prevalence and normal versus abnormal levels of anticardiolipin antibodies (aCL) in a healthy adult population of Omanis and whether a correlation exists between aCL and antinuclear antibodies (ANA) in this Omani population.

**Methods:** A total of 521 healthy Omani individuals (333 males and 188 females), aged between 17-54-years were investigated for the presence and quantities of anticardiolipin antibodies (immunoglobulin G (IgG)) and IgM isotypes using a conventional enzyme linked immunosorbent assay. Antinuclear antibodies were detected, in this group, using standard indirect immunofluorescence techniques. This study was conducted during the period 2002 through to 2003 at the Immunology Laboratories, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman.

**Results:** The prevalence of aCL in the healthy Omani population was estimated to be 2.5% for IgG and 3.1% for IgM. The cut off points for IgG and IgM were determined for the whole population as 22.5 IgG phospholipid (GPL) units and 15.7 IgM phospholipid (MPL) units, using the mean plus 5 standard deviations. Using these cut off values, aCL were not detected in the majority of individuals (97%) and in the remaining 3%, the levels were not very high. There was no significant difference between the levels of aCL in either the male or female groups and no significant correlation for the presence of aCL with the age in this studied population. Antinuclear antibodies were detected in 76/521 (14.6%) of the population studied, with some individuals (0.8%) showing titers of 1:640, but there was no association with aCL.

**Conclusion:** Although, ANA is present in this healthy Omani population at high frequency and in some individuals at high levels, high levels of aCL do not occur and their presence may be an indicator of autoimmune mediated pathology.

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Autoantibodies to phospholipids directed against a variety of anionic phospholipids and plasma proteins are sometimes associated with the antiphospholipid syndromes (APS), which has a wide variety of clinical manifestations. These include recurrent thrombotic events, repeated fetal loss or thrombocytopenia often associated with systemic lupus erythematosus (SLE).[1,2] Usually high levels of phospholipid autoantibodies such as anticardiolipin autoantibodies (aCL), phosphatidylserine autoantibodies and lupus anticoagulant are found in patients with APS and SLE, as well as in drug induced disorders, infectious and neurological diseases.[3-7] Anticardiolipin antibodies may also occur in healthy individuals[8-10] possibly associated with aging.[11,12] To quantitate the risk associated with aCL, it is essential to have accurate information on their prevalence in healthy populations.[10]

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Antinuclear antibodies (ANA) have been detected in many autoimmune disorders and their presence is important for the diagnosis of such disorders, for example SLE.[13] Antinuclear antibodies have been reported to associate with elevated aCL,[14,15] and also known to occur in healthy population.[16]

The aim of the present study was to investigate the prevalence and normal versus abnormal levels of aCL in a healthy adult population in Oman and whether a correlation exist between aCL and ANA in this Omani population.

**Methods.** A total of 521 healthy Omani adults (333 males and 188 females) from different parts of Oman, aged between 17-54 years were studied. Individuals who were less than 17-years-old were excluded from the present study. All individuals had no history or symptoms of autoimmune diseases and no one was taking any medications for chronic diseases. Individuals who have been previously refused to donate blood were excluded from the study as well as pregnant women. Each participant was asked to fill a structured questionnaire. The ethical approval was obtained from Sultan Qaboos University Ethics and Research Committee. A venous blood (10 ml) from each individual was allowed to clot at room temperature for few hours and the serum was separated, divided into aliquots and kept frozen at -20$^{o}$C until thawed at the time of testing.

Anticardiolipin antibodies were measured according to the manufactures instructions, using an enzyme linked immunosorbent assay (ELISA) procedure [Innova Quanta Life™ aCL, IgG, IgM (AP)] with isotype specific antibody to determine the levels of IgG and IgM binding to cardiolipin. Isotype concentrations are expressed as IgG phospholipid (GPL) or IgM phospholipid (MPL) units. One unit represents the binding activity of 1 μg/ml of affinity purified aCL. All serum samples were tested in duplicate and positive samples were repeated. For ANA, sera were screened by indirect immunofluorescence (Quantafluor, Kallested, United States of America) at a 1:20 dilution using HEp-2 cells as the substrate. Positive sera were titrated to determine the end point staining. Positive samples for ANA were retested for antibodies to double stranded DNA (dsDNA) and to extractable nuclear antigens (ENA) using Quanta Life ™ dsDNA, 70851 and Quanta Life ™ ENA ELISA, 708615.

Data analysis was performed using computer program (SPSS 9.0 for Windows). The statistical analysis of cut off points and the significant levels of aCL for the Omani population were determined as described by other investigators.[8-10]

**Results.** **Table 1** shows the cut off values (COV) for aCL and was determined to be 15.7 MPL and 22.5 GPL for the whole population. For males COV were determined as 15.8 MPL, 23.4 GPL while females COV was 11.9 MPL, 15 GPL. Only females showed similar results to the commercial kit with suggested COV for IgM as 12 MPL and IgG as 15 GPL.

The large majority of individuals (approximately 97%) demonstrated undetectable levels of aCL. A total of 16/521 (3.1%) and 13/521 (2.5%) Omanis had raised levels of anticardiolipin IgM and IgG. The maximal aCL level detected in this population was 34.5 GPL for IgG and 20.3 MPL for IgM. **Table 1** shows the descriptive statistics of aCL positive individuals and **Table 2** shows the age distribution of the healthy Omani individuals. The majority of the Omanis were in the 20's and the average age was determined as 25 years. Only 2 males were above the age of 50 years. The age ranges for males was 18-54 years and for females was 17-48 years. There was no evidence of any correlation between aCL and age or gender in this studied Omani population. Antinuclear antibodies were detected in this population in 76/521 (14.6%). The different patterns of ANA detected in this Omani population is shown in **Table 3**. The majority of Omani individuals positive for ANA were shown to have either nucleolar or speckled patterns. Other ANA patterns such as homogenous, rim or centromere, were rarely detected in this population. Four Omani male individuals (0.8%) showed high ANA titers of 1:640 (2 speckled and 2 nucleolar pattern). None of the female volunteers showed titers of ≥1:640. However, 4 females showed titers of 1:160 (2 speckled, one nucleolar and one rim pattern). **Table 4** shows positive samples of ANA, dsDNA and ENA. Only 5 samples (3 males and 2 females) showed levels slightly above the COV for dsDNA and 9 samples (5 males and 4 females) for extractable nuclear antigen (ENA). No significant correlation of aCL and ANA was detected.

**Discussion.** Measurement of lupus anticoagulant, IgG, IgM and IgA cardiolipin autoantibodies by ELISA is the standard procedure for the detection of antiphospholipid antibodies in patients with suspected antiphospholipid syndromes.[1,7,14] Combine testing for phosphatidylserine autoantibodies and lupus anticoagulant in addition to aCL improves the sensitivity for the detection of antiphospholipid antibodies.[17,18] A high aCL concentrations are associated with increased risk of venous and arterial thrombosis, recurrent pregnancy loss and thrombocytopenia.[2,4,18]

**Table 1 -** Descriptive statistics for the anticardiolipin positive Omani individuals.

| Measure | Males | | Females | | Both | |
|---|---|---|---|---|---|---|
| | IgM | IgG | IgM | IgG | IgM | IgG |
| Number positives | 9 | 9 | 7 | 4 | 16 | 13 |
| Percentage | 2.7 | 2.7 | 3.7 | 2.1 | 3.1 | 2.5 |
| Mean | 5.8 | 7.4 | 5.6 | 6.3 | 5.7 | 7 |
| Median | 5.7 | 6.5 | 4.7 | 5.1 | 5.5 | 6 |
| SD | 2 | 3.2 | 2.1 | 2.9 | 2 | 3.1 |
| Maximum | 19.5 | 34.5 | 20.3 | 25.3 | 20.3 | 34.5 |
| Minimum | 2 | 3 | 2.1 | 2.9 | 2 | 3 |
| Age range (years) | 18-54 | | 17-48 | | 17.54 | |
| COV | 15.8 | 23.4 | 11.9 | 15 | 15.7 | 22.5 |
| **Total number** | **333** | | **188** | | **521** | |

Results for IgG and IgM are detected semi quantitively in standard IgG or IgM anticardiolipin units, GPL - IgG phospholipid, MPL - IgM phospholipid, SD - standard deviation, COV - cut off value, IgM - immunoglobulin M, IgG - immunnoglobulin G

**Table 2 -** Age distributon of the Omani healthy individuals.

| Gender | Age (years) | | | | | |
|---|---|---|---|---|---|---|
| | **<20** | **21-30** | **31-40** | **41-50** | **>51** | **Total** |
| Males | 60 | 181 | 68 | 22 | 2 | **333** |
| Females | 78 | 93 | 10 | 7 | 0 | **188** |
| **Total** | **138** | **274** | **78** | **29** | **2** | **521** |

Range is 17-54-years and the average for the whole cohort is 25-years

Although it has been suggested that autoantibodies, in healthy individuals play a physiological role,[19] they may also play a pathogenic roles in autoimmune disorders. As the occurrence of autoantibodies are not restricted to autoimmune diseases,[16] the definition of normal versus abnormal levels of autoantibodies has become more important than ever.[20] Usually abnormal COV for various autoantibodies are poorly defined[9] and the literature revealed cut off points of only between 2-3 standard deviation (SD) of the mean (or median) as the upper limit of normal. This is in addition to the fact that mean and medians are based on only very small healthy control groups. Some studies used the 95% confidence intervals that usually gave a high incidence of positive results. Thus, some commercial laboratories, when evaluating aCL, base their COV only on few control subjects per run. This is in addition to the fact that, COV supplied by the manufacturer of the equipment or the reagents used in quantities that may not reflect the normal values of the local population. In our study, we defined the upper limit of normal as the 99% confidence intervals rather than the 95% confidence intervals based on median for the aCL. Our population of 521 healthy individuals is by far larger than many other studies and using the mean plus 5 SD is more accurate in defining the normal versus abnormal values of aCL in the healthy Omani population.[9,10] Patient levels of aCL below 5 SD of the mean have been shown to have no clinical significance.[21] This enabled us to comment on the incidence and range of these aCL and define accurately their abnormal levels in clinically asymptomatic Omani patients.

As reported by other investigators and our own results, aCL are not normally distributed and therefore nonparametric methods of statistical analysis are necessary to determine population prevalence. Our study revealed only less than 3% of the Omani population having detectable level of

**Table 3** - Different patterns of antinuclear antibodies detected in the healthy Omani population with their titers.

| Antinuclear antibodies pattern | Males (n=333) | | | Females (n=188) | | | Both (N=521) | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | % | Titer range | n | % | Titer range | n | % | Titer range |
| Nucleolar | 22 | (6.6) | 1:40-1:640 | 13 | (6.9) | 1:40-1:160 | 35 | (6.7) | 1:40-1:640 |
| Speckled | 21 | (6.3) | 1:40-1:640 | 15 | (8) | 1:40-1:160 | 36 | (6.9) | 1:40-1:640 |
| Homogenous | 1 | (0.3) | 1:40 | 2 | (1.1) | 1:40-1:80 | 1 | (0.2) | 1:40-1:160 |
| Centromere | 1 | (0.3) | 1:160 | 0 | (0) | 0 | 3 | (0.6) | 1:160 |
| Rim | 0 | (0) | 0 | 1 | (0.5) | 1:160 | 1 | (0.2) | 1:160 |
| **Total** | **45** | **(13.5)** | | **31** | **(16.5)** | | **76** | **(14.6)** | |

**Table 4 -** The occurence of antinuclear antibodies and antibodies to double stranded DNA and extractable nuclear antigens.

| Autoantibody | Males (n=333) | | Females (n=188) | | Both (N=521) | |
|---|---|---|---|---|---|---|
| | n | (%) | n | (%) | n | (%) |
| ANA | 45 | (6) | 31 | (16.5) | 76 | (14.6) |
| dsDNA | 3 | (0.9) | 2 | (1.1) | 5 | (0.95) |
| ENA | 5 | (1.5) | 4 | (2.1) | 9 | (1.7) |

ANA - antinuclear antibodies, dsDNA - double stranded DNA, ENA - extractable nuclear antigens

aCL and these levels were not high and may be considered intermediate. The majority of Omani individuals show levels of aCL below the COV for this population. This study actually suggests that even healthy control populations may harbor a pool of individuals with either subclinical or preclinical autoimmune disease. This is especially important for the identification of women who may be predisposed to reproductive failure.[9,19] For patients with established autoimmune diseases, the clinical symptomatology is usually associated with higher autoantibody levels than states of clinical remission. Therefore, it would be expected that clinically asymptomatic patients who suffer from reproductive failure and found to have abnormal aCL values will exhibit these abnormalities to a lesser degree than symptomatic patients with autoimmune diseases. Only long term follow up of individuals with abnormal values of aCL will determine the pathologic significance of these autoantibodies.

Our sample of the Omani population represent both gender and an age distribution from 17-54 years of age. Within this age distribution, there was no evidence of any association between aCL levels and either gender or age. There is no clear indication from this study that the distribution of aCL in individuals over the age of 50 differs from individuals under 50, for only 2 male individuals were above the age of 50-years.

Antinuclear antibodies were detected in 14.6% of the studied population, a much higher level than reported previously.[16] We have examined the correlation between the presence of ANA and the low levels of aCL detected. No correlation, whatsoever, was detected between ANA and aCL in this study. Moreover, the different patterns of ANA detected in this population were nucleolar and speckled and there was no significant difference in the presence of either in the male or female individuals. Other patterns of ANA were rarely seen in this Omani population. Different ANA patterns are usually associated with different autoimmune diseases. The fact that 4 Omani male individuals had titers of 1:640 and 4 females had titers of 1:160 may indicate an autoimmune mediated pathology and only long term follow up of these persons may confirm this suspicion. We have observed that more female volunteers have ANA. The exact explanation for this observation is not clear to us at present, but may be related to the female predominance of certain autoimmune diseases, such as SLE.

The existence of autoreactive antibodies, both in healthy subjects and in patients with autoimmune diseases is a consequence of intra thymic selective processes during the development of the T-cell repertoire.[22] Exposure of self antigens to the thymic environment during fetal life, results in the elimination of specific anti self T-cells, the dominant mechanism is physical, that is, clonal deletion.[22] Autoreactive T-cells against antigens which do not pass through the thymus during developmental stages may be eliminated via functional inactivation namely, clonal anergy. Failure of either of these processes will result in the maturation of autoreactive T-cells. There are many mechanisms which result in the breakdown of self tolerance. They include alteration in the control of apoptosis, cross reactivity and molecular mimicry, antiidiotype antibodies that function as autoantibodies and the polyclonal stimulation of natural autoantibody producing cells that then progress via mutation and isotype switching.[22] Autoimmunity is readily inducible by drugs or infectious agents and potentially reversible, namely, it may disappear when the offending drug or agent is withdrawn.[22]

Although we have demonstrated that aCL IgM and IgG are not very common among the healthy adult Omani population, cautions must be used in basing clinical decisions on the presence of these antibodies alone. Moreover, the screening test for aCL is recommended as a supportive method for prenatal follow up of pregnant patients.[20]

We hope that by establishing the normal versus abnormal aCL levels in this Omani population will facilitate a more accurate definition of clinically asymptomatic patients with abnormal autoimmunity. Given that abnormal autoimmune function has been widely implicated in the reproductive failure,[20,23] this study should have an impact on the diagnosis, treatment and follow up of such patients.

In conclusion, this study has shown that ANA may be present at high levels and in some healthy Omani individuals at high titers. High levels of aCL do not occur and their presence may be an indicator of autoimmune mediated pathology.

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