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Antiphospholipid antibodies in Mexican HIV-positive patients

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ABSTRACT

Several studies have shown that HIV patients tend to develop autoimmune diseases, and have numerous antibodies, such as antiphospholipid antibodies. Antiphospholipid antibodies are the serological markers used in the diagnosis of the antiphospholipid syndrome. However, antiphospholipid antibodies also appear to exist in infectious diseases.

Objective: To measure the titers of antiphospholipid antibodies in healthy and in HIV positive Mexican mestizo patients, and correlate them with the patient clinical manifestations to identify possible findings compatible with an autoimmune disease.

Material and methods: A case control study was conducted on 50 healthy mixed race Mexican subjects and in 50 randomly selected HIV-positive patients from the Infectious Diseases Department of a Regional Hospital in Puebla, México. Antiphospholipid titers were performed on the patients and controls and analyzed to see if there was any correlation between clinical signs.

Results: There was a statistical difference in the titers of anticardiolipin antibodies isotype IgG between the control group and the HIV group. When sexual preference was evaluated in the HIV group a statistical difference in the antibody titers was observed between homosexual and heterosexual HIV patients.

Conclusion: There were no correlations found between the antibody titers and specific clinical manifestations in HIV positive patients. The exact clinical meaning of the presence of these antibodies in HIV positive patients is still unknown, so further studies are needed.

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Anticuerpos antifosfolípido en pacientes mexicanos VIH positivos

Palabras clave:
VIH
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RESUMEN

Varios estudios han demostrado que los pacientes con VIH tienden a desarrollar enfermedades autoinmunes, presentando diversos anticuerpos como los anticuerpos antifosfolípidos. Los anticuerpos antifosfolípido son los marcadores serológicos que se emplean en el diagnóstico del síndrome antifosfolípido. Sin embargo los anticuerpos antifosfolípido también suelen existir en enfermedades infecciosas específicas.

Objetivo: Medir los títulos de anticuerpos antifosfolípido en pacientes mexicanos mestizos sanos y VIH positivos y correlacionarlos con las manifestaciones clínicas de los pacientes para identificar posibles hallazgos compatibles con alguna enfermedad autoinmune.

Material y métodos: Se trata de un estudio de casos y controles en el que se evaluaron los títulos medios de anticuerpos antifosfolípidos en 50 mestizos mexicanos mestizos sanos y 50 VIH positivos, así como su correlación con las manifestaciones clínicas de cada paciente, siendo éstos elegidos aleatoriamente del servicio de Infectología de un Hospital Regional en Puebla, México.

Resultados: Hubo una diferencia estadísticamente significativa en los títulos de anticuerpos anticardiolipina del isotipo IgG entre el grupo control y el grupo infectado por VIH. Cuando se evaluó la preferencia sexual en el grupo de los pacientes VIH positivos se encontró una diferencia estadísticamente significativa en los títulos de anticuerpos anticardiolipina entre pacientes homosexuales y heterosexuales con VIH.

Conclusiones: No se encontró ninguna correlación entre los títulos de anticuerpos y las manifestaciones clínicas en los pacientes infectados con VIH. El significado exacto de la presencia de estos anticuerpos en los pacientes infectados por VIH continúa siendo desconocido, por lo que se necesitan más estudios en el futuro.

Introduction

Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies directed against negatively charged phospholipids, protein–phospholipid complexes and phospholipid binding proteins measured by solid-phase immunoassays.[1,2] These antibodies are detectable in serum of patients with various diseases including autoimmune diseases, lymphoproliferative disorders, coagulation disorders, recurrent fetal loss, and some viral and bacterial infections, secondary to an intense antigenic stimulation of the immune system.[3–5]

aPL such as lupic anticoagulant (LA), anticardiolipin (aCL) and anti β-2-glycoprotein (aβ2-GPI) antibodies are associated with an increased risk of arterial and venous thromboembolism, which is a common clinical finding in the antiphospholipid syndrome (APS).[2,3] The aβ2-GPI is not required to establish the diagnosis of APS; however, it has been linked to thrombotic events.[6] The aPL are related to a prothrombotic condition due to modifications in the coagulation pathway, associated to recurrent pregnancy morbidity such as fetal loss and thromboembolic complications.[2,3]

aPL have also been associated to viral and bacterial infections. The first description was with syphilis, but many other viral, bacterial and parasitic infections have also shown to increase aPL, mainly aCL. With the wide use of the aCL ELISA for the detection of aPL in the diagnosis of the APS, it was quickly noticed that this test is also regularly positive in patients with viral and bacterial infections.[7] aPL have been detected in serum of patients infected with Hepatitis C Virus, Human Immunodeficiency Virus (HIV), Cytomegalovirus, Varicella Zoster Virus, Epstein–Barr Virus, Adenovirus, Parvovirus B 19, legionnaires diseases, Tuberculosis, among others.[4,8]

Specifically aCL and aβ2-GPI have been found increased in patients with chronic HIV infections, but their association with thrombotic events has not been proven.[9] Abuaf et al. reported on the prevalence of aPL in HIV infection, and aCL were reported to be present in 0–94%, aβ2-GP1 in 4–47%.[10,11] aβ2-GPI are not usually found in this type of patients; besides when they are absent or exist at low titers there is a minor risk for a thrombotic events to occur.[4,9,12] Some authors have proposed the association between these antibodies and the development of symptoms; therefore, it is important to reevaluate the real role of these antibodies in HIV infection and to determine if it is an epiphenomenon of no clinical relevance.

In HIV infection, aPL have been closely linked with viral replication levels. On one hand, some authors suggest that HIV infection is associated with many abnormalities in B cell function, resulting in the production of a large variety of autoantibodies. Therefore, in HIV positive patients, aPL, specifically aCL, are considered to be a marker of impaired humoral immunity.[4] On the other hand, Solis et al., in a case report, showed that as the viral load increased, the aPL titers increased too.[5] This could definitely support the idea that stronger antigen stimulation involves a stronger immune response, even though it is a faulty one.

APS is a disease characterized by thrombotic events and several laboratory abnormalities.[2,6] It usually affects arterial and venous beds causing transient ischemic attacks, stroke or deep venous thrombosis, respectively.[3] The diagnosis is

established by the presence of aCL IgG or IgM isotypes at medium or high titers in two or more measurements 12 weeks apart or the presence of LA plus clinical evidence of vascular thrombosis, confirmed by Doppler or histopathology tests or the presence of pregnancy morbidity such as fetal loss or premature births. It is interesting to note that many authors have found aCL IgG and IgM isotypes in serum of patients with HIV no matter their sex, risk factors and stage of disease, but still the diagnosis of APS is not commonly integrated in these patients.

The clinical significance of these antibodies in infection is controversial because these antibodies are not pathogenic, and do not appear to be of an autoimmune nature, what is justified by the absence of thrombotic events. It is a fact that thromboembolic events are reported in approximately one-third of HIV positive patients with aPL, but again, a few HIV positive patients have been diagnosed with APS. The primary objective of this study was to determine mean titers of aPL in HIV positive patients and analyze their correlation with the patient clinical manifestations mainly thrombotic/ischemic.

Patients and methods

Patients

The presence of aCL antibodies in HIV positive individuals was determined in fifty Mexican mestizo patients selected among those attending the Infectology Service at a Regional Hospital No 36, during the period from March 2009 to June 2009. All of them previously signed an informed consent; the protocol was authorized by the Hospital Ethics Committee. HIV positive patients (12 females and 38 males) had an age range of 22–67 years. Fifty HIV negative individuals (8 females and 42 males) had an age range of 25–52 years.

5 ml of peripheral blood was obtained from each individual, the blood was centrifuged in order to obtain the serum; the serum from healthy individuals was obtained from the hospital's Blood Transfusion Service. Serum was stored at −40 °C until tested and was not re-frozen.

Methods

A case control study was conducted to compare the titers of aPL detected from serum of HIV positive and negative patients. aPL specifically, aCL of IgG and IgM isotypes and β2-GPI, were measured using a commercial enzyme immunoassay (ELISA) kit (Bindazyme, the Binding Site Ltd., Birmingham, UK) and results were expressed in units, according to the manufacturer instruction. To increase the precision we analyzed all samples by duplicate, and performed a calibration curve of 5 points according to international guidelines.[13]

Statistical analysis

Quantitative variable means were expressed as means ± standard deviation. Comparison of values between the case and control subjects was determined using Fisher's exact test. Spearman's rank correlations were used for comparisons of antibody levels between patient groups. The statistical analysis was performed using the GraphPad Prism software.

Results

The mean age of the 50 Mexican HIV positive patients was 41.94 ± 11.81 years. The sexual preference of HIV patients was 46% heterosexual, 34% homosexual, and 20% bisexual. All the individuals who formed the control group were heterosexual. Patients were not selected; the Infectology service and Blood Transfusion Service sent consecutively, only those patients who agreed to participate, without asking their sexual preferences. Before taking the blood sample, we asked all individuals to fill out a questionnaire; the data analysis showed that there was an important difference in the sexual preferences of HIV patients attending the regional hospital No. 36 in Puebla compared to the control group, because in this we realized a bibliographic search and found that some authors had published differences in some molecules depending on the sexual preferences, for this reason we decided to take into account the sexuality of our participants to perform the antiphospholipid level analysis.

The data collected from the medical records as expected were very diverse; we made a classification of the most common symptoms in our group, and diarrhea (34%), anemia (20%), skin manifestations (15%), urinary tract infection (12%), Hepatitis B Virus infection (10%) and lower respiratory tract infection (10%) were the more common findings. 6% of the patients had bone pain, herpes simplex virus infection, warts, sore throat, peripheral polyneuropathy, pharyngitis, myopathy, and neuro-infection.

The evaluation of aPL showed that aCL IgG levels were significantly higher than those found in control individuals. The mean titer of aCL IgG in the HIV patients was 16.18 ± 41.37 U/ml, compared to control group 3.076 ± 2.002 U/ml ($p = 0.0001$). 10% of patients showed high positive aCL titers, the mean level in these patients was 134.160 ± 39.961 U/ml. When the aCL IgG levels were analyzed by sexual preference we found a statistically significant difference between individuals whose sexual preference was homosexual to those heterosexual ($p = 0.025$) (Table 1).

The analysis of aCL IgM showed that the mean titer in HIV patients was 5.47 ± 12.92 U/ml and in the control group it was 2.38 ± 2.20 U/ml, but there was no statistically significant difference. 6% of the patients had high positive levels of aCL IgM (83.9 ± 2.57), but when the group was analyzed by sexual preference there were no differences between groups.

The mean titer of aβ2-GPI in our HIV population was 2.55 ± 2.21 U/ml, and in the control group was 2.1 ± 1.1 U/ml, both groups showed normal levels of aβ2-GPI. All these results are shown in Table 1.

Discussion and conclusion

Our results showed that in the HIV group, the aPL, specifically aCL IgG or IgM isotypes were present, and were found in higher titers than the control group. As shown in Table 1, the data analysis showed important differences in the aPL levels between HIV patients and the control group, as we mention in

Table 1 – The levels of aPL (U/ml) in our study groups when the sexual preferences were analyzed.

	Heterosexual	Homosexual	Bisexual	HIV Group	Control
aCL IgG[a]	3.63 ± 4.33	32.5 ± 59.3	13.8 ± 35.8	16.18 ± 41.3	3.076 ± 2.002
aCLIgM[b]	4.60 ± 10.0	8.59 ± 18.1	3.30 ± 2.86	5.47 ± 12.91	2.38 ± 2.20
aβ2-GPI[c]	3.58 ± 4.78	2.75 ± 2.0	2.31 ± 1.02	2.509 ± 2.21	2.1 ± 1.1

[a] Ig G anticardiolipin antibodies.
[b] Ig M anticardiolipin antibodies.
[c] Anti-β2 glycoprotein antibodies.

the results section, there was a statistical difference in the aCL IgG levels between these groups, although no statistical difference was found when the HIV group was analyzed by sexual preferences, but it is very important to emphasize that the aCL IgG is 10 times higher in homosexual patients and 4 times higher in the bisexual compared to the control group. Also it is interesting that those heterosexual HIV patients had the same IgG levels than the control group. Unfortunately this is not statistically significant because of the large standard deviation present in our group. We had also interesting results with the aCL IgM levels; in this case, the differences were minor although homosexual HIV patients had 3.6 times more IgM levels than control.

The clinical records were analyzed and the data obtained demonstrated that sexual preference had an apparent relation with higher antibody titers. There are reports in the literature such as Canoso et al. that shows an increment of IgG aCL in homosexual men or the report of Mulhall who showed that 57% of homosexual have an increase of aPL.

Although the levels of aCL of the IgG isotype was significantly increased in the patients, there are no clinical data on these patients that correlate with antiphospholipid antibodies novel, so this elevation of antibodies may be only an epiphenomenon rather associated with the infection process and that has nothing to do with the process inflammation present in the APS. The levels of these antibodies were higher in those homosexual individuals with HIV, may be because they are associated with other infections acquired previously; as reported in the literature this group have a higher rate of infections.

At the moment of the study, no patient had a diagnosis of APS nor had any isolated thrombotic event. APS is known to present other spectrum of clinical manifestations like thrombocytopenia, myelitis transverse, movement disorders and livedo reticularis but none of these were present in any patient who had high titers of aPL.[2,3,6,16] No correlations were found between aPL titers and other clinical manifestations or the drugs used for their treatment.[17] Clinical manifestations can be found in advanced stages of AIDS, in the form of microangiopathic thrombosis of the glomerulus.[12] Several hypotheses have been proposed trying to explain the asymptomatic thrombosis or renal failure in HIV positive patients with aPL; some of them propose primary endothelial cell damage with liberation of vasoactive molecules such as the vasoconstrictor endothelin-1, IL-6, and TNF-α.[2,4]

The reported prevalence of aPL levels in infections is very variable, and this can be due to different methodological procedures used for their quantification, such as the type of assay used, the different cut-off levels that indicate positivity of the test, the heat applied for the activation of serum, among others. Patient selection and ethnic diversity may also play a major role in the discrepancies found for aPL positivity reported in infections such as HIV, syphilis and HCV.

Currently clinicians are not aware of the presence of aPL in a diversity of diseases. Although the role of aPL in HIV is contradictory there are reports of no association[14–16] or positive association between having positive aCL and thrombosis,[17,18] so it is important to define clearly if the levels of aPL influence the clinical presentation of antiphospholipid syndrome in HIV patients. Therefore the detection of aPL in different clinical situations could help to avoid future thrombotic events, improving the prevention with prophylaxis and the general outcome.

Responsabiliadades éticas

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of Data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study have received sufficient information and have given their informed consent in writing to participate in that study.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and/or subjects mentioned in the article. The author for correspondence is in possession of this document.

Conflict of interest

Authors state that there is no conflict of interest of any kind.

REFERENCES

1. Santiago M, Martinelli R, Mittermayer G, Almeida E, Ko A, Dias R, et al. Frequency of antiphospholipid antibodies in patients with infectious diseases. Using three different ELISA methods. J Bras Pathol Med Lab. 2006;42:13–7.
2. Levine J, Branch W, Rauch J. The antiphospholipid syndrome. N Engl J Med. 2002;346:752–63.

3. Hanly J. Antiphospholipid syndrome: an overview. CMAJ. 2003;168:1675–82.
4. Asherson RA, Cervera R. Antiphospholipid antibodies and infections. Ann Rheum Dis. 2003;62:388–93.
5. Solis J, Gómez J, Fernandez M. Antiphospholipid syndrome and acute HIV infection. Emerg Infect Dis. 2010;16:360–1.
6. Mackworth-Young CG. Antiphospholipid syndrome: multiple mechanisms. Clin Exp Immunol. 2004;136:393–401.
7. Forastiero R. Antigen specificity of antiphospholipid syndrome-related antiphospholipid antibodies. Open Autoimmun. 2010;2:21–7.
8. Gharavi AE, Pierangeli SS. Infections and antiphospholipid antibodies. In: Khamashta MA, editor. Hughes syndrome: antiphospholipid syndrome. Great Britain: Springer; 2000. p. 135–43.
9. Asherson R, Shoenfeld Y. Human immunodeficiency virus infection. antiphospholipid antibodies, and the antiphospholipid syndrome. J Rheumatol. 2003;30:214–9.
10. Becker AC, Sliwa K, Singh S, Stewart S, Tikly M, et al. Antiphospholipid antibodies in black South Africans with HIV and acute coronary syndromes: prevalence and clinical correlates. BMC Res Notes. 2011;4:379.
11. Abuaf N, Laperche S, Rajoely B, Carsique R, Deschamps A, Rouquette AM, et al. Autoantibodies to phospholipids and to the coagulation proteins in AIDS. Thromb Haemost. 1997;77:856–61.
12. Nuñez A, Gavela E. Fracaso Renal en Paciente VIH. Nefrología. 2004;24:97–100;
Derksen RHWM, de Groot PG. Clinical consequences of antiphospholipid antibodies. Nederlands J Med. 2004;62:273–8.
13. Harris EN, Pierangeli SS. Revisiting the anticardiolipin tets and its standardization. Lupus. 2002;11:269–75;
Sedlacek D, Ulcová-Gallová Z, Milichovská L, Nováková P, Rokyta Z. Seven antiphospholipid antibodies in HIV positive patients: correlation with clinical course and laboratory findings. Amer J ReprodImmunol. 2003;50: 439–43.
14. Canoso RT, Zon LL, Groopman JE. Anticardiolipin antibodies associated with HTLV-III infection. Br J Haematol. 1987;65:495–8;
Uthman I, Gharavi A. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum. 2002;31: 256–63.
15. Mulhall BP, Naselli G, Whittingham S. Anticardiolipin antibodies in homosexual men: prevalence and lack of association with human immunodeficiency virus (HIV) infection. J Clin Immunol. 1989;9:208–13;
Galrao L, Brites C, Atta ML, Lima I, Gonzalez F, Magalhaes F, et al. Antiphospholipid in HIV positive patients. Clin Rheumatol. 2007;26:1825–30.
16. Palomo I, ALarcón M, Sepulveda C, Pereira J, Espinola R, Pierangeli S. Prevalence of antiphospholipid and antiplatelet antibodies in human immunodeficiency virus (HIV) infected Chilean patients. J Clin Lab Anal. 2003;17: 209–15.
17. Leder AN, Flansbaum B, Zandman-Gooldard G, Asherson R, Shoenfeld Y. Antiphospholipid syndrome induced by HIV. Lupus. 2001;10:370–4.
18. Uthaman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum. 2002;31:256–63.