Anti Annexin A5 antibodies in patients with unexplained recurrent early pregnancy loss

Thesis
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List of Abbreviations

aCL……anticardiolipin antibodies
AnxV……..annexin V
APCR…….activated protein C resistance
APS……antiphospholipid syndrome
aPS…….antiphosphatidylserine
aPL ……Antiphospholipid antibodies
β2GPI ……B2 Glycoprotien 1
CV …….Coefficients of variation
CVA ……..Cerebrovascular accident
D.M ……...Diabetes Mellitus
DVT ……...Deep vein thrombosis
ELISA…… enzyme immunometric assay
EPCR……..Endothelial protein C receptor
G A …….Gestational age
G S ……. Gestational sac
HCG……humane chorionic gonadotropines
HELLP……syndrome hoemolysis, elevated liver function test, Low platelet count
HUVEC……..human umbilical vein endothelial cell cultures
IGFBP-1…….insulin like growth factor binding protein
INR…….international normalized ratio
IUGR……..intrauterine growth retardation
IVF-ET…….. in vitro fertilization/embryo transfer
LA ……..lupus anticoagulant
LDA ……. .Low-dose aspirin
Ml ……..Myocardial infarction
No. ……number
PAPI ……..placental anticoagulant protein I
PEA…….. .phosphatidylethanolamine
PKC …….….protein kinase C
PS ……..phosphatidylserine
RSPL……..Recurrent spontaneous pregnancy loss
RSA……....recurrent spontaneous abortion
SLE……....systemic lupus erythromatosis
SSc…….systemic sclerosis
STAT5……...single transducer and activator of transcription 5
VACa…….vascular anti-coagulant a

Abstract

The APS is an autoimmune disorder in which patients have antibodies to phospholipids or phospholipidbinding proteins (aPL) in combination with clinical manifestations such as vascular thrombosis and/or recurrent pregnancy losses. There are conflicting reports on the association of anti-annexin A5 antibodies with RSPL and reproductive failure in humans (***Levine et al, 2002)***.

The objective of our study was to determine any association between the anti annexin V Ab IgG isotope and unexplained recurrent spontaneous abortion.

Methods: 50 women were included in the study. They were divided in to two groups: GROUP 1 patient group: included 25 cases that had a history of at least two or more successive unexplained recurrent abortion in their first trimester. GROUP 2 control group: included 25 cases that had at least two successful pregnancies and no history of pregnancy loss.

Results: We found that 10 cases of the 25 patients were positive for IgG ANX V Ab (40%), in contrast for 7 controls of 25 women found positive for IgG ANX V Ab (28%), and we found that 15 cases of 25 patients were positive (60%), in contrast for 8 controls of 15 women were found positive for IgM ANX Ab (53.3%).

Conclusion: Anti annexin A5 antibody of class IgG or IgM measured before the beginning of pregnancy are not predictive of the outcome of the next pregnancy.

Key words: APS, aPL, RSPL, ANX V Ab IgG, ANX Ab IgM.

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Introduction

Abortion is the most frequent complication of pregnancy, with an incidence ranging between 8% and 25%, depending on the population studied and the data collection method ***(Regan & Rai, 2000)***.

Pregnancy loss after implantation is frequent and idiopathic recurrent spontaneous abortion (RSA) is often seen in otherwise healthy women ***(Mahjoub et al., 2005).***

Many factors are considered potentially liable to cause abortion: chromosomal abnormalities, uterine abnormalities, hormonal imbalances, coagulation defects, and infectious and immunological causes. In general, however, it is considered that chromosomal abnormalities are strongly prevalent before the 8th week of gestation and that a high percentage of the miscarriages occurring after the 8th week of gestation may have immunological causes ***(Huang et al., 2005)***.

The physiologic adaptation during pregnancy appears to be related to the development of an adequate placental circulation and provide a protective mechanism for homeostasis during delivery. The increase in procoagulant factors generates a hypercoagulable state and consequently increases thrombotic risk during pregnancy and the immediate postpartum period. The risk is higher in women with thrombophilic defect ***(Bonnar et al., 1999)***.

Thrombophilia is a multigentic disorder caused by

inherited and acquired defects and can be defined as a predisposing to thrombosis. Antiphospholipid antibodies (aPL) are recognized as one of the most important causes of acquired thrombophilia ***(Ana et al., 2002)***.

Anti-phospholipids antibodies (aPL) are a heterogeneous group of autoantibodies. The persistent presence at medium-high levels of which is associated with the occurrence of thromboembolic events and fetal loss. ***(Wilson et al., 1999)***.

The anti-phospholipid syndrome is defined as the association of venous and\or arterial thrombosis, thrombocytopenia, and the production of autoantibodies against negatively charged phospholipids which are clinically associated with reproductive failure (repeated pregnancy loss, either early or late, intrauterine growth retardation, prematurity, placental abruption, induced hypertension in pregnancy, uteroplacental insufficiency, HELLP syndrome – hoemolysis, elevated liver function test, Low platelet count) ***(Rand et al., 2005).***

APL pregnancies are characterized by defective endovascular trophoblastic invasion of maternal uterine spiral arteries, extra villous trophoblast invasion being normal. Also aPL affect the expression of markers-prolactin and insulin like growth factor binding protein (IGFBP-1) of decidulisation, whish is the process by whish the endometrial stromal cells are transformed in the second half of menstrual cycle in readiness for implantation. aPL also has a direct inhibitory effect on the STAT5(single transducer and activator of transcription 5),intracellular singling pathway which is involved in the expression of genes controlling deciduation ***(Rand et al,2003).***

Annexin A5, which was known as annexin V, a potent anticoagulant, was isolated and cloned from human umbilical cord arteries and placenta in the mid 1980s. It is composed of 319 amino acids. It is highly expressed by the apical surfaces of the syncytiotrophoblasts, which face the maternal circulation, and other cell types that serve as barrier function between tissues and body fluids ***(Van Heerde et al., 2004).***

The potent anticoagulant properties of the protein result from that it is forming highly ordered 2-dimensional crystals that coat the external leaflets of phospholipids bilayers that shield them from availability for critical phospholipid-dependent coagulation reactions, which is an ideal anatomic position to promote blood fluidity in the uteroplacental circulation. Also it has been suggested that annexin V forms an antithrombotic shield around procoagulant anionic phospholipids, such as phosphatidylserine (PS) calcium dependent, which blocks their participation in phospholipid-dependent coagulation reactions, and that the binding of specific high-affinity antibodies to annexin V can compromise the antithrombotic shield and contribute to reproductive failure ***(Rand et al., 2004).***

It has been demonstrated by atomic force microscopy that annexin V binding to phospholipids or its anticoagulant activity was significantly reduced by plasmas of patients with antiphospholipid antibodies and thrombosis compared with healthy controls and patients with thromboembolism and without antiphospholipid antibodies ***(Rote et al., 2002)***.

In addition, monoclonal antibodies against annexin V block intertrophoblastic fusion, suggesting another possible mechanism whereby these antibodies may induce miscarriage ***(Rote et al., 2002)***.

Anti-AnxV antibodies were present in 17% of subjects with recurrent miscarriage and were the only immunological marker among aCL, anti- β2GPI, and antiprothrombin, which was correlated with the occurrence of miscarriage, though to a moderate extent ***(Bizzaro et al., 2005)***.

During embryonic and placental differentiation, a disruption of the lipid asymmetry occurs, leading to exposure of PS on the outer surface during the apoptotic processes accompanying the trophoblast differentiation. Blockage of PS by Abs hinders syncytium formation, thus enforcing apoptosis. Women with reproductive failure were found to have elevated levels of circulating aPS Abs associated with complications of Pregnancy ***(Rote & Stetzer, 2003)***.

An association between the presence of antiphosphatidylserine (aPS) and complication of pregnancy was documented in women with APS or pregnancy loss. This pathogenicity could be explained by more than one mechanism ***(Manno et al., 2002)***, for example:

a) Shading the phosphatidylserine on the trophoblast cells interferes with signaling which leads to reduction in hCG production and invasion of the extracellular matrix to form the syncytiotrophoblast;
b) Prevention of annexin-V binding to PS which was shown to restrict intrauterian growth ***(Manno et al., 2002).***

These mechanisms indicate that the presence of these autoimmune factors involving production of antibodies targeting phospholipids (cardiolipin and phosphatidylserine) or phospholipid-binding proteins (b2GPI and annexin V)) are closely related to RSA, via a mechanism which requires participation of immune and nonimmune factors in precipitating a prothrombotic state that favors pregnancy loss ***(Walid Zammiti,et al., 2006).***

Aim of the work

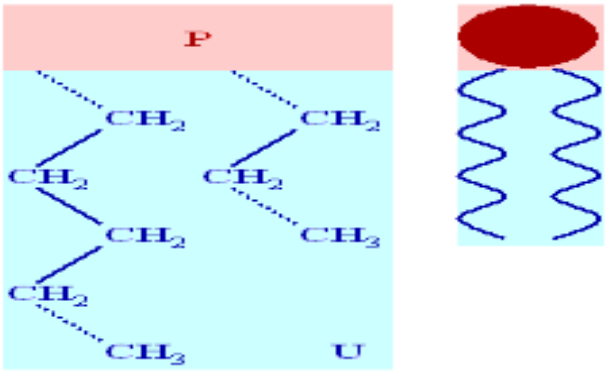
The aim of the work is to estimate the level of Anti annexin A5 antibody, in the sera of unexplained recurrent pregnancy loss patients, and to find the relation between them.

THE ANTIPHOSPHOLIPID SYNDROME

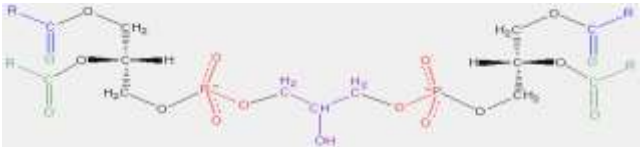
PHOSPHOLIPIDS

Phospholipids are the primary components of cellular membranes. Each molecule contains a hydrophilic phosphate head and two hydrophobic fatty acid tails. This is important because phospholipids self assemble in water into a bilayer ***(Wilson et al, 1999)***.

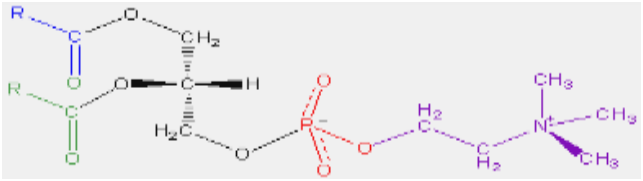
The phospholipids head of the molecule determines the charge and function of molecule. Phospholipids can be anionic (negative charge, diphosphatidylglycerol [cardiolipin], phosphatidylserine, and phosphatidylglycerol), cationic (positive charge phosphatidylinositol and phosphatidic acid), or neutral charge (phosphatidylethanolamine and phophatidylcholine). ***(Mayes et al., 1993).***



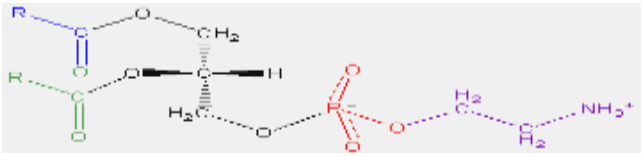
Phospholipids structure *(Mayes et al., 1993).*



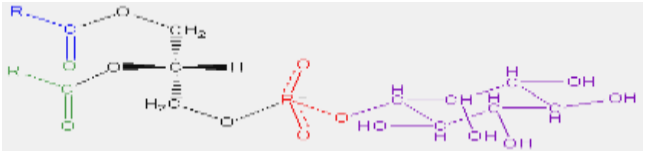
Diphosphatidyl-Glycerol



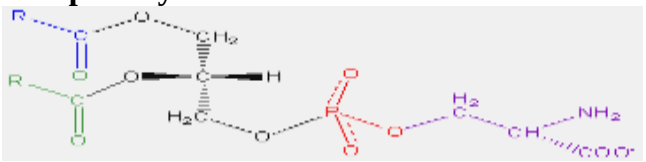
Phosphatidyl-Choline



Phosphatidyl-Ethanolamine



PhosphatidylInositol



Phosphatidyl-Serine

Figure (1)**:** Phospholipids structure and phospholipids ***(Mayes et al., 1993).***