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Antiphospholipid syndrome and abortion :Immunological study

Hameed M. Naif Shihab A. Lafi RaJaa J. Al- hadethi

University of Anbar - College of Medicine.

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ABSTRACT

This study was conducted to detect the presence of antiphospholipid antibodies in the blood samples collected from one hundred and four women who were suffering from pregnancy loss (abortion) via detection of Anticardiolipin IgG and IgM using Enzyme Linked Immunosorbant Assay(ELISA). Increased autoantibodies (ACL) levels in women that had spontaneous abortion which anticardiolipin antibodies (ACL) levels in the serum of aborted women compared with control groups (twenty healthy pregnant and non pregnant women). Eleven (10,57%) out of 104 women were showed positive results for ACL. Three (2,88%) of them had positive results for ACL IgG and other eight (7,79%) have positive results for ACL IgM . The high frequency of positive results were includes within the age group (31-35). Four (36.36%) women out of eleven who had positive results for anticardiolipin test showed mild thrombocytopenia. Antinuclear antibody test and Anti Ds.DNA test were performed using ELISA to exclude Systemic Lupus Erythematosus (SLE), all results for these tests were negative. Anti toxoplasma antibodies (IgM) were found in (4) (3,84%) from test group women. Anti CMV antibodies type (IgM) were found in (5) (4,8%) from test group women. Anti Rubella antibodies type (IgM) were found in (4) (3,84%) from total aborted women. Anti (HSV 1,2) antibodies type (IgM) found in only one (0,96%) from total aborted women. women who gave positive results for above infection were excluded.

Introduction

The antiphospholipid syndrome (APS) is a systemic autoimmune disturbance differentiated by a group of arterial and / or venous thrombosis, recurrent pregnancy loss, often accompanied by a mild-to-moderate thrombocytopenia, and high titers of antiphospholipid antibodies (APL), namely the lupus anticoagulant (LA) and / or anticardiolipin antibodies (ACL). [1, 2]

Excessive blood clotting and/or recurrent miscarriage in early pregnancy and other complications of pregnancy involving preterm labour, pre-eclampsia and low birth weight fetal death beyond the tenth week of gestation are the most typical obstetric complications of APS and the presence of antiphospholipid antibodies (cardiolipin or lupus anticoagulant antibodies) in the blood.[3] Approximately 15% of women with recurrent miscarriage have persistently positive tests for either LA or ACL, compared to 2% of those with an uncomplicated obstetric history[4,5] .The majority of miscarriages among women with APL occur between seventh and twelfth weeks of gestation and represent the loss of chromosomally normal fetuses [6]. The antiphospholipid syndrome can be classified into several categories. "Primary" antiphospholipid syndrome occurs in patients without clinical evidence of other autoimmune disease, and "secondary" antiphospholipid syndrome occurs in association with autoimmune or other diseases. Since systemic lupus erythematosus (SLE) is by far the most common disease with which the

* Corresponding author at: University of Anbar-College of Medicine. E-mail address: hamid_pio06@yahoo.com

antiphospholipid syndrome occurs [7]. Catastrophic antiphospholipid syndrome is a variant of antiphospholipid syndrome that is characterized by blockage of many blood vessels throughout the body. As a result of catastrophic antiphospholipid syndrome, many organs can be affected, including the skin, lungs, brain, heart, kidneys, and bowels [8]. Harmless antiphospholipid antibodies can be diagnosed in the blood for a short period occasionally in association with a wide variety of conditions, involving bacterial, viral (hepatitis, HIV), and parasite (malaria) infections. The drugs can cause antiphospholipid antibodies to be produced in the blood, such as antibiotics , cocaine, hydralazine, procainamide, and quinine [9]. The antiphospholipid antibody (a protein) is not considered a normal blood protein and has been found to be associated with a number of illnesses . These illnesses include abnormal clotting (thrombosis) of arteries (stroke, infarction) and/or veins ,miscarriages (spontaneous abortions), abnormally low platelet counts (thrombocytopenia), purplish mottling discoloration of the skin (livedo reticularis). migraine headaches, and a rare form of inflammation of the nervous tissue of the brain or spinal cord, called transverse myelitis [9 ,10].This study was devoted to study the rate of antiphspholipid syndrome among abortion cases in Ramadi city, West of Iraq and to study of more important tests or parameters for these cases and determination the level of Tumor Necrosis Factor – alpha (TNF-α) in the serum of women with antiphospholipid syndrome.

Patients and methods:

Study group:

One hundred and four (104)women with history of pregnancy loss were included in this study .These women were attended Maternity and Child Teaching Hospital in Ramadi during the period extended from December 2011 to June 2012. Each woman involved in the study was examined by senior obstetrician to exclude medical and anatomical cause of abortion and all inclusion criteria recommended for their cases were applied.

Control group :

This group included twenty(20) intact women who had no history of abortion (pregnant and non pregnant women) .Each woman was examined and manipulated in the same manner of women in the study group.

Blood Specimens:

Five milliliters (5ml) of venous blood were taken aseptically from each women . One milliliter (1ml) of blood was kept in EDTA tube for platelets count according to Bain et al (2012)[11] . The rest of each blood specimen was transferred to a plastic tube without anticoagulant and used for serum pooling . Serum was separated by centrifugation at 3500 RPM for 10 min. Pooled serum samples were kept in Ependrof tube at -20 C° to be used for serological investigation.

Serological tests :

Other causes of abortion were excluded through performing TOARCH test to exclude Toxoplasma , Rubella and Herpes Simplex virus 1,2 (HSV1,2. infections using special ELISA kits for these investigations (Biocheck kit, USA) .

Sera of patients were tested for the presence of Aticardiolipin IgG and anticardiolipin IgM using AntiCardiolipin IgG/ IgM ELISA kit of (Orgentec Diagnostika GmbH., Germany).

Enzyme Immunoassay for qualitative screening of antinuclear antibodies and quantitative determination of IgG antibodies to Double Stranded DNA (ds- DNA) were done to exclude SLE using AESKU, Diagnostic GMBH Mickroforum, (Germany). Tumor necrosis Alpha was detected in sera of patients using Tumor Necrosis Factor-alpha TNF-α ELISA Demeditec Diagnostic GMBH Germany kit.

Statistical Analysis

Data of this study were analyzed using Microsoft Excel XP. Chi-square test and T test was used wherever appropriate and probability value less than 0.05 ($P<0.05$) was considered to indicate statistical significance.

Results:

The mean of platelets count in patients with antiphospholipid syndrome (APS) was lower than the mean of platelets in patients without APS and the mean of platelets in control group (figure-1). Four patients out of eleven who had positive results for anticardiolipin had mild to moderate thrombocytopenia in this study. Anticardiolipin antibodies test results showed 11 (10.57%) had positive results out of 104 women with history of abortion. Three patients showed positive results for Anticardiolipin IgG, they were located within age group (21-25), (26-30) and(31-35). The total percentage of women who showed positive results for anticardiolipin IgG test was (2.8%) while the percentage of women who have negative result for this test was (97.2%)(101) (Table-2, Fig-2). Anticardiolipin IgM test results showed that Eight (7.7%) out of 104 women gave positive results for this test while (96)(92.3%) out of 104 women showed negative results for this test . Among patients group only women within age group (16_20) years showed negative anticardiolipin IgM while the patients showed positive anticardiolipin IgM, four of them (50%) found within age group (31-35) and other patients were located within age group (21-25), (26-30), (36-40) and (41-45) (Table- 3). Higher rate of abortion was found in women within age group (21-25) and (26-30) years and majority of women were showing abortion found within the first months of gestation(Fig.3) All patients within all age groups gave negative results for Antinuclear antibodies and Anti Ds.DNA test (Table-4a and 4b).All patients within all age groups showed negative results for Tumor Necrosis Factor alpha test (Table-5a,table-5b).There was no significant correlation between the syndrome and serum TNF- α. titer test $p < 0.05$.

Discussion

Antiphospholipid syndrome is systemic autoimmune disorder, It is caused by family of autoantibodies called Antiphospholipid antibodies [(12)] . These antibodies lead to cause venous and/or arterial thromboses, pregnancy loss (including fetal death and recurrent pre-embryonic and embryonic losses), pre-eclampsia, premature delivery due to pregnancy-associated hypertensive disease and placental insufficiency often accompanied by a mild-to-moderate thrombocytopenia,There are several hypotheses to explain the role of antiphospholipid antibodies in the pathogenesis of recurrent miscarriage most of these assumptions adopted on thrombus formation that prevent the passage nutrients to the fetus leading to fetal death , Another hypothesis was suggested that antibodies cause insufficiency in the work of trophoblast cells in the placenta leading to reduce secretion of human chorionic gonadotropin which is necessary for the continuation of the pregnancy[(13)]. Lower platelets count was found in patients with positive anticardiolipin IgG and IgM ,this was ought to consumption of platelets in the previous clotting process in placenta leading to fetal loss and antibodies destroy them[(14,15)].So women with obstetric antiphospholipid syndrome were at high risk of subsequent thrombotic complications [(16)] The most common kinds of antiphospholipid antibodies are the anticardiolipin antibodies.[(17)] Anticardiolipin antibodies (ACL) were measured through searching for these antibodies in form of IgG or IgM. Prevalence of antiphospholipid syndrome among general population is low (1- 4.5%) and it was increased with age [(18)] . Other reports showed that number of individuals in the general population with positive antiphospholipid antibodies in their blood range was from 2% [(19)] to 6.5%. [(20)]**.** In this study results indicated that 11(10.57%) of women with pregnancy loss had positive results for anticardiolipin antibodies IgG and IgM. This result was not in accordance with findings of (Kadhim, 2007)[(21)] who showed that 27% of aborted women ,27% of them had positive results for anticardiolipin IgG and IgM in their sera . This might be due to differences in the sample number and age of patients . The elevated anticardiolipin antibodies IgG titer may increase the risk of thrombosis more than five times and the specificity of anticardiolipin antibodies for diagnosis of antiphosoholipid syndrome increases with higher titer of IgG [(22)]. No positive result was found in the age group (16-21) that may be due to numbers of women in these age group were small and APS increased with age .

Four positive results were found in the age group (31-35)This may be due to APS increased with age [(18, 23)]. Majority of women had fetal loss in the first month of gestation , this was not in accordance with the

findings of Gharavi and Wilson (1996) and Out *et al* (1991) [19, 24]. Fetal loss in such females might be due to the effect of antiphospholipid antibodies on implantation of zygote [25], as well as the role of blood clotting in placental vessels leading to placental damage [13] . TORCH test was used to exclude other reasons of abortion like infection with *Toxoplasma gondii* ,Rubella virus ,Cytomegalo virus and Herpes simplex virus-type-2 and patients gave positive results were excluded from the study. The antinuclear antibodies test (ANA) was used to differentiate between primary and secondary antiphospholipid APS. Regarding SLE test in this study non of women in test and control groups showed positive results for this test. This indicated that patients who had positive results for anticardiolipin were not related to other autoimmune diseases and they have primary antiphospholipid syndrome .[26,27] The antidouble stranded DNA test used to differentiate between the abortion caused by antiphospholipid antibodies and abortion caused by other autoimmune disease such as systemic lupus erythematosus. In this study non of women in study group and control group showed positive results for this test , this indicated the patients who have positive results for anticardiolipin antibodies test have antiphospholipid syndrome not another autoimmune disease . This results confirm our finding that non of women were affected with SLE. Negative results for Anti Double stranded DNA and Antinuclear antibodies in sera of test and control groups indicated correct inclusion and exclusion criteria for test and control women included in this study. All sera of test and control women were negative for TNF-α ,this may be due to local (in placental than in peripheral blood) TNF- α release , therefore no increase TNF-α level in peripheral blood was observed and this factor may undergo increase locally in placenta in pregnant females with atiphospholipid syndrome [28] .

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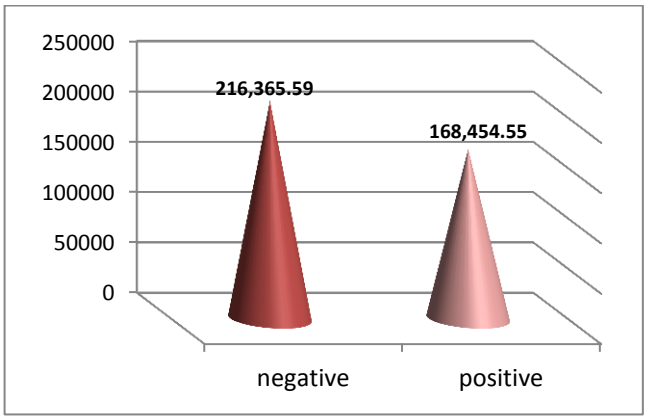

Figure -1: The mean of platelets count for patients with APS and other without APS.

Table -2 : Relationship between abortion and anticardiolipin IgG

Parameters	Anticardiolipin IgG test results		Total	Chi- test	P value
	Positive	negative			
Patients	3	101	104	26.117	0.00

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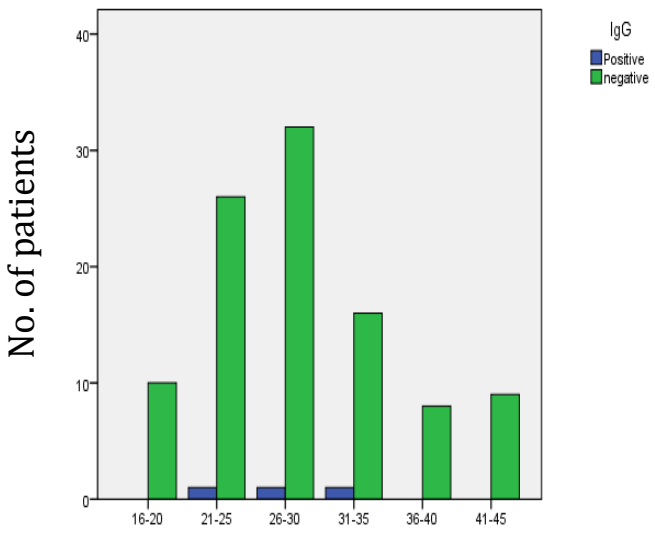

Figure-2:Age groups and anticardiolipin IgG test

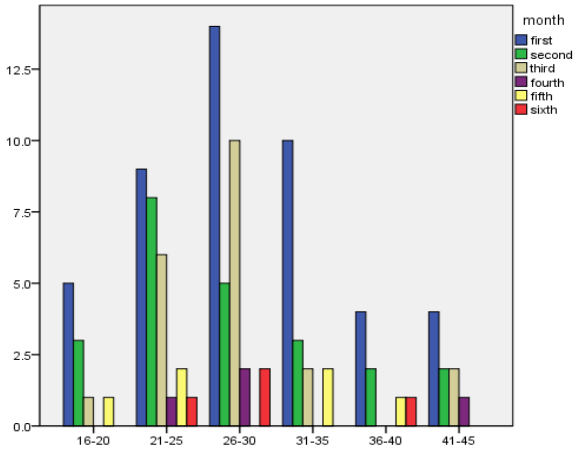

Figure -3: Age group and month of pregnancy loss

Table -3: Age group and Anticardiolipin IgM test

Age group		Anticardiolipin IgM		Total
		Positive	negative	
16-20	No. of patients	0	10	10
	%	.0%	10.4%	9.6%
21-25	No. of patients	1	26	27
	%	12.5%	27.1%	26.0%
26-30	No. of patients	1	32	33
	%	12.5%	33.3%	31.7%
31-35	No. of patients	4	13	17
	%	50.0%	13.5%	16.3%
36-40	No. of patients	1	7	8
	%	12.5%	7.3%	7.7%
41-45	No. of patients	1	8	9
	%	12.5%	8.3%	8.7%
Total	No. of patients	8	96	104
	%	100.0%	100.0%	100.0%

Relationship between abortion and antiDs.DNA test
Table-4:

Parameters	Anticardiolipin Antibodies test results	Anti Ds.DNA antibodies test	Total
		negative	
Patients	positive	11	11
	negative	93	93
Total		104	104

Table .5a: Results of Tumor Necrosis Factor alpha among age groups of test study .

Age group		T.N.F.α test	Total
		Negative	
16-20	No. of patients	10	10
	%	9.6%	9.6%
21-25	No. of patients	27	27
	%	26.0%	26.0%
26-30	No. of patients	33	33
	%	31.7%	31.7%
31-35	No. of patients	17	17
	%	16.3%	16.3%
36-40	No. of patients	8	8
	%	7.7%	7.7%
41-45	No. of patients	9	9
	%	8.7%	8.7%
Total	No. of patients	104	104
	%	100.0%	100.0%

Table -5b: Relationship between abortion and T.N.F-α

Parameters	Anticardiolipin Antibodies test results	Tumor Necrosis factor alpha	Total
		negative	
Patients	positive	11	11
	negative	93	93
Total		104	104

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متلازمة أضداد الدهون الفوسفاتية والإجهاض: دراسة مناعية.

حميد محمود نايف شهاب احمد لافي رجاء جهاد الحديثي

Email: hamid_pio06@yahoo.com

الخلاصة:

أجريت هذه الدراسة للكشف عن وجود الأجسام المضادة (APL)في عينات الدم التي تم جمعها من 104 من النساء اللاتي عانين من فقدان الحمل (الإجهاض) عبر الكشف عن الأجسام المضادة للشحم القلبي(ACL)(IgG و IgM) بواسطة الفحص المناعي المرتبط بالأنزيم (ELISA) أظهرت هذه الدراسة زيادة في مستويات الأجسام المضادة للشحم القلبي في النساء اللاتي يعانين من اجهاضات عفوية مقارنة بمجموعة السيطرة التي ضمت نساء حوامل وغير حوامل. احد عشر (10.57%) من هؤلاء النساء أظهرت نتائج ايجابية لاختبار ألأجسام المضادة للشحم القلبي (ACL) ، ثلاثة (2.88%) منهن لديهن نتائج ايجابية لاختبار IgG (ACL) وثمانية (7.7%) أخريات لديهن نتيجة ايجابية لاختبار IgM (ACL) وهناك وتيرة عالية من النتائج الإيجابية تتركز في الفئة العمرية (26–30). أربعة من إحدى عشر من النساء اللاتي لديهن نتائج ايجابية لاختبار الأجسام المضادة للشحم القلبي ظهرت لديهن قلة في الصفيحات الدموية.اختبار الأجسام المضادة للنواة واختبار الأجسام المضادة للشريط المزدوج من الحامض النووي الرايبوسومي منقوص الأوكسجين بواسطة الفحص المناعي المرتبط إنزيميا التي تمت في هذه الدراسة ، فإن جميع النتائج سلبية لكلا الإختبارين مما يعطي دليل واضح على أن الإجهاض في النساء ذوات النتائج الإيجابية لاختبار (ACL) كان بسبب الأجسام المضادة للشحوم الفوسفاتية وليس بسبب داء الذئب الإحمراري (SLE). تم العثور على أجسام مضادة IgM لطفيلي داء المقوسات Toxoplasma gondii في أربعة(3.83%) حالات من مجموعة الدراسة في حين تم العثور على أجسام مضادة نوع IgM لفيروس المضخم للخلايا في خمس(4.8%) حالات من مجموعة الدراسة.وقد وجدت أجسام مضادة نوع IgM لفيروس الحصبة الألمانية في أربعة (3.84%) حالات من مجموعة الدراسة .في حين تم إيجاد حالة واحدة حاملة لأجسام مضادة للنوع الثاني من فيروس الحميراء البسيط من مجموعة الدراسة. وقد أجريت هذه الاختبارات في هذه الدراسة لاستبعاد أسباب الإجهاض الذي تحدث بسبب هذا الإصابات.