Anticardiolipin Antibodies and Lupus Anticoagulants among Women Undergoing *In Vitro* Fertilization in Jamaica

V DaCosta, L Chin, S Wynter, J Harriott, L Christie, S Frederick-Johnston, J Frederick, C McKenzie, D Everett, R Foster, Y Walters, A Pottinger

ABSTRACT

Objective: *The aims of this study were to evaluate the effect of anticardiolipin antibody (aCL) and lupus anticoagulant (LA) on the outcome of the* in vitro *ferlitization (IVF) cycles and to determine the prevalence of these antibodies in infertile women seeking IVF in Jamaica.*

Methods: *A retrospective cohort study was performed to determine if screening patients for aCL and LA had any significant impact on the outcome of the IVF process. Each patient's hospital record, between March 2000 and March 2010, was collected and the relevant data extracted.*

Results: *The prevalence of aCL in this cohort of Jamaican women was moderate/high positive 3.88%, low positive 0.68% and those with negative aCL results 95.4%. The prevalence of women who were LA positive was 4.1% and 0.9% of the women were positive for both LA and aCL. Of the patients who were LA and/or aCL positive, eight out of 30 patients (26.7%) had a positive pregnancy test in comparison to 61 out of 181 patients (33.7%) who were LA and/or aCL negative (*p = *0.5787).*

Conclusion: *The prevalence of positive aCL and/or LA in infertile women seeking IVF in Jamaica is 7.76%. The presence of these antibodies did not affect the pregnancy rate of these women nor did it demonstrate an increased risk for IVF cycle cancellation or ovarian hyperstimulation syndrome. Screening women undergoing IVF for these antibodies is not justified.*

Keywords: Anticardiolipin antibodies, IVF, Jamaica, lupus anticoagulants

Anticuerpos Anticardiolipinas y Anticoagulantes Lúpicos entre Mujeres a las que se les Realiza FIV en Jamaica

V DaCosta, L Chin, S Wynter, J Harriott, L Christie, S Frederick-Johnston, J Frederick, C McKenzie, D Everett, R Foster, Y Walters, A Pottinger

RESUMEN

Objetivo: *Los objetivos de este estudio fueron evaluar el efecto del anticuerpo anticardiolipina (aCL) y el anticoagulante lúpico (LA) sobre el resultado de los ciclos de la fertilización en vitro (FIV), así como determinar la prevalencia de estos anticuerpos en mujeres estériles que buscan tratamiento de FIV en Jamaica.*

Métodos: *Se realizó un estudio de cohorte para determinar si el tamizaje de pacientes para detectar el anticuerpo anticardiolipina y el anticoagulante lúpico tenía un impacto significativo en el resultado del proceso de FIV. Se obtuvieron las historias clínicas hospitalarias de cada una de las pacientes, entre marzo de 2000 y marzo de 2010, y se extrajeron los datos pertinentes.*

Resultados: *La prevalencia de aCL en esta cohorte de mujeres jamaicanas fue 3.88% moderada/alta positiva, 0.68% positiva baja, y aquellas con resultados negativos de aCL, 95.4%. La prevalencia de mujeres con resultados de anticoagulante lúpico positivos fue 4.1%, y 0.9% de las mujeres resultaron positivas con respecto tanto al LA como al aCL. De las pacientes que fueron positivas al LA y/o al aCL, ocho de cada 30 pacientes (26.7%) tuvieron una prueba de embarazo positiva, en comparación con 61 de cada 181 pacientes (33.7%) negativas al LA y/o al aCL (*p = *0.5787).*

From: Hugh Wynter Fertility Management Unit, The University of the West Indies, Kingston 7, Jamaica, West Indies.

Correspondence: Dr V DaCosta, Hugh Wynter Fertility Management Unit, The University of the West Indies, Kingston 7, Jamaica, West Indies. E-mail: ved111@hotmail.com

Conclusión: *La prevalencia de resultados positivos en relación con anticuerpos anticardiolipinas y/o anticoagulantes lúpicos en mujeres estériles que buscan FIV en Jamaica es 7.76%. La presencia de estos anticuerpos no afectó la tasa de embarazo de estas mujeres, ni mostró un aumento de riesgo de la cancelación del ciclo FIV, o riesgo de síndrome de hiperestimulación ovárica. El tamizaje en busca de estos anticuerpos en mujeres que buscan tratamiento de FIV, no está justificado.*

Palabras claves: Anticuerpos anticardiolipinas, FIV, Jamaica, anticoagulantes lúpicos

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INTRODUCTION

Antiphospholipid syndrome (APS) or the presence of antiphospholipid antibodies (aPLs) and lupus anticoagulant (LA) usually present as pregnancy loss and/or thrombosis. However, aPLs have also been reported to affect implantation, placentation, and early embryonic development. The binding of aPL to beta-2 glycoprotein-1 (GPl) may lead to breakdown of the phospholipid adhesion molecules between different elements of trophoblast (1). As aPLs affect placental growth and function, aPLs may prevent implantation presenting as infertility. The lupus anticoagulant and anticardiolipin antibodies have been implicated in the prothrombotic effects of APS (1). The presence of aPLS among infertile women is likely to be a part of a generalized autoimmune disturbance associated with infertility (2).

If the presence of aPLs or LA alone, without the APS or systemic lupus erythematosus (SLE), does not affect the outcome of assisted reproductive technology (ART), then screening or therapy is not justified.

The Hugh Wynter Fertility Management Unit (HWFMU) at the University of the West Indies is faced with a "high" demand for assisted reproductive techniques from females of all ages and social classes. It is therefore very important that we keep the cost of this procedure as low as possible. One of the ways that we can decrease the patient's expense is to avoid doing unnecessary costly investigations.

The clinical relevance of the aPLs in women undergoing *in vitro* fertilization/embryo transfer (IVF/ET) and the role of IVF treatment in affecting antiphospholipid levels are controversial.

Wharfe *et al* (3) showed that the incidence of anticardiolipin antibody (aCL) in a cohort of Jamaican women with miscarriages was moderate/high positive in 15.2%, low positive in 23.1% and negative in 61.6%. The paper concluded that "the prevalence of positive immunoglobulin-G aCL tests was high in this cohort of patients with spontaneous abortion. However, intervention is not necessary in many of these patients who have only a positive aCL test but none of the clinical conditions of the antiphospholipid syndrome". Other studies have supported this conclusion (4–6).

In a recently published Jamaican study by Roye-Green *et al* (7), blood samples from 50 women who had had recurrent spontaneous abortions and 135 healthy multiparous women were investigated for aCL and anti-β-2 GP1 dependent aCL antibodies by enzyme-linked immunosorbent assays (ELISA). Lupus anticoagulant activity was measured by activated partial thromboplastin time, anti-nuclear antibodies, rheumatoid factors and thyroid antibodies using standard techniques.

There was no significant difference in the prevalence of autoantibodies in habitual aborters and control subjects (60% and 44%, respectively). Habitual aborters differed from controls only in the prevalence of positive aCL tests (15/50, 30% *vs* 15/135, 11%, $p = 0.01$), medium/high concentrations of aCL (9/50, 18% *vs* 9/135, 7%, $p = 0.05$); aCL of the IgM isotype (8/50, 16% *vs* 7/135, 5%, $p = 0.05$) and anti-β-2 GP1 antibodies (7/50, 14% *vs* 3/135, 2%, $p = 0.05$). They recommended aCL screening in habitual aborters and the performance of the anti-β-2 GP1 antibody tests to identify those most at risk.

Some reports have suggested a relationship between aPLs and infertility and poor ART outcome (1, 8). However, recent studies have not detected a higher prevalence of these autoantibodies in infertile women, or a correlation with the type of female infertility diagnosis (9). Another group failed to observe a higher prevalence of LA or aCL in women with unexplained infertility than in a control group of fertile egg donors (10). In addition, the presence of aPLs does not seem to affect ART outcome (11), and therefore therapy is not justified (9). The presence of aPLs among infertile women is likely to be a part of a generalized autoimmune disturbance that may be associated with infertility (2). Hence, routine screening is not warranted in the infertile population and therapeutic interventions are not to be advised, except in the case of women with other features of APS, for example, recurrent miscarriage or pregnancy complications (9, 12).

The aims of this study are to evaluate the effect of anticardiolipin antibody and lupus anticoagulant on the outcome of the IVF cycles at the HWFMU and to determine the prevalence of these antibodies in infertile women seeking IVF at the HWFMU.

SUBJECTS AND METHODS

Between March 2000 and March 2010, all patients attending the HWFMU for IVF with moderate/high positive aCL, those with low positive aCL, and those with positive LA antibody

results were identified, along with randomly assigned controls with similar demography. All patients undergoing IVF gave their consent for the above investigations to be performed.

A retrospective cohort study was performed to determine if screening patients for the aCL and LA had any significant impact on the outcome of the IVF process. Each patient's hospital record was collected and the relevant data extracted (Appendix).

Inclusion criteria for cases included all patients undergoing IVF with a positive aCL and/or positive LA result. Inclusion criteria for controls included all patients undergoing IVF with a negative aCL and negative LA result.

Exclusion criteria for cases included patients with a history of having APS or SLE and patients who were taking steroids or those who discontinued steroids within three months of starting IVF. Exclusion criteria for controls were patients with a history of having APS, SLE, or a positive aCL, or positive LA result.

The statistical package, SPSS version 17, was utilized for data analysis and independent sample *t*-tests and Chi-square statistics were used to compare differences in the outcomes in the two groups and determine statistical significance.

RESULTS

Between March 2000 and March 2010, 438 patients attended the HWFMU for IVF and met the criteria for inclusion in the study. Thirty-four of these patients were positive for LA and/or aCL. Thus, the prevalence of these antibodies in infertile women seeking IVF at the HWFMU was calculated to be 7.76%.

The prevalence of the aCL in this cohort of Jamaican women was moderate/high positive in 3.88%, low positive in 0.68% and negative aCL results in 95.4%. The prevalence of women who were LA positive was 4.1% and 0.9% of the women were positive for both LA and aCL.

The demographic data of age, height, weight and body mass index (BMI) were found to be similar between the two groups with no statistical differences (Table 1).

The most common cause of infertility among the women presenting for IVF was due to a male factor (45%). This was followed by tubal disease (31.5%), endometriosis (10.5%), uterine factors (4.6%), unexplained factors (4.6%) and ovarian factors (3.8%) [Figure].

The means of the following variables for those patients who were aCL and/or LA negative and positive were analysed: duration of infertility, total number of antral follicles (2–10 mm in size), day three luteinizing hormone (LH), follicle stimulating hormone (FSH) and oestradiol levels, number of follicles measuring greater than 14 mm at the time of stimulation with human chorionic gonadotrophin (hCG),

Table 1: Demographic characteristics of the patients

	Lupus and/or anticardiolipin status						*p*-value
	Negative			Positive			
	N	Mean	SD	N	Mean	SD	
Age (years)	204.0	35.2	4.8	34.0	35.6	5.1	0.6
Weight (kg)	202.0	71.8	14.1	34.0	74.4	15.0	0.2
Height (m)	202.0	1.6	0.1	34.0	1.7	0.1	0.4
Body mass index (kg/m^2)	200.0	26.5	4.9	34.0	27.3	5.4	0.6

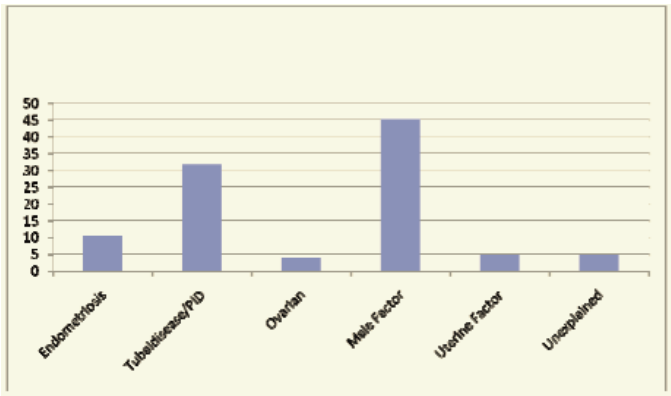

Figure: Causes of infertility among women presenting for *in vitro* fertilization at the Hugh Wynter Fertility Management Unit.

number of oocytes retrieved, number of oocytes fertilized, total number of embryos obtained, and the number of embryos transferred. There was no statistical difference between the two groups [Table 2]. Of the patients who were LA and/or aCL positive, four out of 34 patients (11.8%) had cancellation of their IVF cycle in comparison to 26 out of 205 patients (12.7%) who were LA and/or aCL negative [Table 3]. This difference was found to be statistically significant using Chi-square analysis ($p = 0.02$).

With regards to a positive pregnancy test, of the patients who were LA and/or aCL positive, eight out 30 patients (26.7%) had a positive pregnancy test in comparison to 61 out of 181 patients (33.7%) who were LA and/or aCL negative [Table 4]. This difference was not statistically significant using Chi-square analysis ($p = 0.5787$).

With regards to the occurrence of ovarian hyperstimulation syndrome (OHSS), of the patients who were LA and/or aCL positive, two out 31 patients (6.5%) developed OHSS in comparison to 12 out of 174 patients (6.9%) who were LA and/or aCL negative [Table 5]. This difference was statistically significant using Chi-square analysis ($p = 0.0082$).

Table 2: Data obtained for lupus anticoagulant (LA) and/or anticardiolipin antibody (aCL) positive patients *versus* LA and/or aCL negative patients

Variable	Lupus anticoagulant and anticardiolipin status								*p*-value
	Negative				Positive				
	N	Mean	min	Max	N	Mean	min	max	
Duration of infertility (years)	201	4	1	20	34	3	1	15	0.3
LH (IU/L)	203	4	1	25	34	4	1	14	0.7
FSH (IU/L)	204	6	1	17	34	7	1	24	0.7
Oestradiol (pmol/ml)	198	51	0	386	33	54	1	170	0.5
Number of antral follicles	201	4	0	45	34	3	0	16	0.7
Number of follicles >14 mm at the time of stimulation	201	8	0	32	34	7	1	20	0.2
Oocytes retrieved	192	9	0	42	32	9	2	23	0.7
Oocytes fertilized	188	5	0	19	31	4	0	13	0.8
Total embryos obtained	186	4	0	16	30	4	1	13	0.3
Total embyros transferred	187	2	0	6	30	3	1	5	0.3
Total number of transfer procedures	187	1	0	2	30	1	0	2	0.8

LH = luteinizing hormone; FSH = follicle stimulating hormone

Table 3: Cancellation of *in vitro* fertilization (IVF) cycles with respect to lupus anticoagulant and anticardiolipin status

Cycle cancelled	Lupus anticoagulant and anticardiolipin status		Total
	Negative	Positive	
No	179	30	209
Yes	26	4	30
Total	205	34	239

Chi-square *p*-value = 0.02

Table 4: Positive pregnancy test results with respect to lupus anticoagulant and anticardiolipin status

Positive pregnancy Test	Lupus anticoagulant and anticardiolipin status		Total
	Negative	Positive	
No	120	22	142
Yes	61	8	69
Total	181	30	211

Chi-square *p*-value = 0.5787

Table 5: Occurrence of ovarian hyperstimulation syndrome (OHSS) in patients with respect to lupus anticoagulant and anticardiolipin status

OHSS	Lupus anticoagulant and anticardiolipin status		Total
	Negative	Positive	
No	162 (93.1%)	29 (93.5%)	191 (93.2%)
Yes	12 (6.9%)	2 (6.5%)	14 (6.8%)
Total	174	31	205

Chi-square *p*-value = 0.0082

DISCUSSION

The analysis of this study revealed that the prevalence of LA and aCLs (7.76%) was low in this cohort of women. There were no significant differences between the two groups in clinical characteristics such as age, infertility duration, and response to controlled ovarian hyperstimulation. The prevalence in this study is much lower than that reported by the Practice Committee of the American Society for Reproductive Medicine (12), and Chilcott *et al* (13). This prevalence is also lower than those reported by Wharfe *et al* (3) and Roye-Green *et al* (7), two other Jamaican studies. The lower prevalence of LA and aCLs in this study may be related to the high prevalence of male infertility (Figure) in the study population.

This study also showed that pregnancy rate, live birth rate, gestational age at delivery and birthweight were not affected by the presence of aPL and/or LA in patients undergoing IVF. This is in agreement with Bellver *et al* (10) and a meta-analysis by Horstein *et al* (11).

The prevalence of the aCL in this cohort of Jamaican women was moderate/high positive 3.88%, low positive 0.68% and negative aCL results 95.4%. These figures are

much lower than those reported in previous studies on cohorts of Jamaican women with histories of habitual abortion (3, 7). Previous evaluation of aCL levels and the effect of IVF treatment on aCL in women undergoing their first IVF/ET cycle indicated that IVF treatment increased IgG-aCL levels in patients with endometriosis and tubal factor infertility, but their presence seemed to have no clinical relevance or effect on the IVF cycle outcome (14).

This study demonstrated no difference in the percentage of patients who achieved a biochemical pregnancy among the women who tested negative and those who tested positive. This is in contrast to a previous study which analysed the influence of maternal immunological factors on clinical pregnancy outcome in an IVF-ET programme (15). One hundred and fifty IVF-ET treatment cycles were studied. A lower pregnancy rate (9.5%) was found in the aPL-positive group, compared with 26.3% in seronegative women ($p < 0.05$). They concluded that serum immunological factors may play a part in clinical pregnancy outcome in IVF-ET. However, more recent studies (2, 13) have refuted this finding and report similar findings with the present study.

A prospective study published in 2007 by Sanmarco *et al* (15) assessed the prevalence of aPLs in women who had undergone IVF and the relationship between aPLs and IVF outcome. All were evaluated for the presence of LA, antibodies (IgG, IgA, IgM) to cardiolipin (aCL), anti-β-2 GP1 and phosphatidylethanolamine (aPE). Out of 101 infertile women, 40 were persistently positive for aPL, showing a prevalence significantly higher than in controls (39.6% *vs* 5%, $p < 0.0001$). Among aPL, aPE was found with a significantly higher prevalence compared with LA, aCL, and aB2GPI (67.5% *vs* 0%, 15%, and 40%, respectively). The study by Sanmarco *et al* (15) demonstrated aPE as the most prevalent aPL in infertile women and the results did not support an association between aPL and IVF outcome.

The present study also demonstrated that there was no increased risk for cycle cancellation or the development of the OHSS among women who were LA or aCL antibody positive. These findings support the recommendation to eliminate these costly investigations from the evaluation of the infertile couple presenting for IVF. Limitations of this study stemmed from noted deficiencies in documentation which would have allowed for further analysis of pregnancy outcomes. The majority of the patients returned to their referring obstetrician-gynaecologist after their positive pregnancy test, and were lost to follow-up, thus precluding analysis of the live birth rate.

CONCLUSION

Approximately 8% of the infertile women seeking IVF at the HWFMU over the ten-year period studied were aCL and/or LA positive. The presence of these antibodies did not affect the pregnancy rate of these women nor did it demonstrate an increased risk for an IVF cycle cancellation or OHSS. Screening women undergoing IVF for these anti-bodies is not justified. Thus, there is the potential benefit from a health economics viewpoint in eliminating these un-necessary investigations and their associated cost.

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APPENDIX: ABSTRACTION FORM

Age
Parity
Years of infertility
Cause of infertility
Lupus anticoagulant
Moderate/high positive aCL
Low positive aCL
Long protocol
Microdose flare cycle
Number. of follicles
Number of eggs collected
Number of embryos transferred
Number of fertilizations
Positive pregnancy test
Clinical pregnancy
Ovarian hyperstimulation syndrome