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Anticardiolipin antibodies in Jamaican primiparae

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Summary

A prevalence survey of anticardiolipin antibodies (aCL) was done among 924 primiparae. aCL were measured in serum by the standardised anticardiolipin enzyme linked immunosorbent assays (ELISA) and β_2-glycoprotein 1 assays to determine the seroprevalence of both β_2 glycoprotein 1 dependent aCL and β_2-glycoprotein 1 independent aCL in Jamaican primiparae, to determine whether aCL are associated with abnormal pregnancy outcomes and if treatment with aspirin had any effect on pregnancy outcome in aCL positive primiparae. The prevalence of aCL was (32/671) 4.8% (95%CI 3.2–6.4) in women who were tested twice. A total of 49 of 924 primiparae or 5.3% (95%CI 3.9–6.7) were positive for aCL on at least one occasion. Only three of the 32 primiparae 3/32 (9.4%) who were positive for aCL on two occasions were positive for β_2-glycoprotein 1 dependent aCL. Pregnancy outcome did not differ significantly with respect to aCL status. Aspirin therapy did not influence pregnancy outcome in the 49 aCL positive primiparae studied.

Introduction

Anticardiolipin antibodies (aCL) are a group of autoantibodies also known as antiphospholipid antibodies (APL) (Harris 1990a). aCL are associated with recurrent spontaneous abortion, pre eclampsia, intrauterine growth retardation, low birth weight, preterm deliveries and fetal distress (Reece et al. 1990 Yasuda et al. 1995) as well as thromboembolic phenomena (Kahwa et al. 2001). aCL related pregnancy complications are believed to be due to placental insufficiency resulting from clotting in placental vessels which interferes with gas exchange and nutrient supply to the fetus leading to intrauterine growth retardation and fetal death (Out et al. 1991; Levy et al. 1998; Gharavi et al. 2001).

High prevalence of IgG aCL was reported in a group of Jamaican women with spontaneous abortion (Wharfe et al. 2004). However, the prevalence and impact of aCL among pregnant Jamaican women is not known. The prevalence of pre-eclampsia reported in residents of the parish of Kingston and St. Andrew, Jamaica was 7.2% (Golding 1998) and low birth weight was estimated at 10% of all live births (Thomas et al. 1989). This study sought to determine the seroprevalence aCL in normal Jamaican primiparae, whether aCL are associated with abnormal pregnancy outcomes and if treatment with low dose aspirin (60 mg) had any effect on pregnancy outcome in aCL positive primiparae. The results of this study should inform the usefulness of routine screening for aCL in pregnancy in Jamaica.

Materials and methods

Subjects

A total of 924 of 6,275 (14.7%) primiparae who participated in the Jamaica Low Dose Aspirin Trial (Golding 1998) and consented to phlebotomy were enrolled in the Anticardiolipin Antibody Study. Subjects enrolled in the Jamaica Low Dose Trial were residents of the parishes of Kingston and St Andrew and were between 12 and 32 weeks' gestation at the time of enrolment.

On enrolment in the aspirin trial, subjects were randomly assigned to aspirin treatment (60 mg) or placebo. Of the 924 women enrolled in the Anticardiolipin Antibody Study 505/1,863 (27.1%) were enrolled from the University Hospital of the West Indies Antenatal Clinic, 231/682 (33.8%) from the Kiwanis Maternity Center and 188/454 (41.4%) were enrolled from the Comprehensive Health Center Antenatal Clinic. Only primiparae who gave informed consent and donated a blood sample for anticardiolipin antibody testing were enrolled. Ethical approval for the study was obtained from Ethical Committees of both the University Hospital of the West Indies (UHWI)/ University of and the West Indies (UWI) and the Ministry of Health, Jamaica.

Definitions of abnormal pregnancy outcomes

A term delivery was defined as a delivery after 37 or more completed weeks of pregnancy. A pre-term delivery was defined as a delivery before 37 weeks. A spontaneous abortion was defined as the premature natural expulsion of the products of conception from the uterus, i.e. an embryo or non-viable fetus before 24 weeks' gestation. A stillbirth was defined as fetal death *in utero* of 24 or more weeks' gestation. Such a product of conception failed to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from uterus, with no possibility of resuscitation. Low birth weight was defined as birth weight of $<2{,}500$ g. Pre-eclampsia was defined as pregnancy induced hypertension with proteinuria of one

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plus (+) or greater. Eclampsia was defined as convulsions (fits) occurring in a pregnant or puerperal woman associated with pre-eclampsia. Pregnancy outcomes were documented as part of the Jamaica Low Dose Aspirin Trial (Golding 1998) using four standardised questionnaires: enrollment, interim, delivery and follow-up questionnaires.

Measurement of anticardiolipin antibodies

Two blood samples were collected from 671, while 253 primiparae donated only one blood sample. The first sample was collected at booking, while the second sample was collected in the third trimester. Serum was separated, aliquoted and stored at −70°C until tested. aCL were detected in serum using anticardiolipin enzyme-linked immunosorbent assays (ELISA) described by Harris (Harris 1990b; Kahwa et al. 2001). A calibration curve for each assay was constructed using calibrators supplied by the Antiphospholipid Standardization Laboratory (Louisville, Kentucky, USA) and the concentration of aCL in serum samples was computed in reference to this curve using the BIO-RAD software (BIO-RAD Laboratories, Hercules, California, USA). aCL of the IgA, IgG and IgM isotypes were determined and quantified in IgA antiphospholipid (APL) units, IgG antiphospholipid (GPL) units and IgM antiphospholipid (MPL) units, respectively. aCL concentration of <10 units was considered negative, low positive 10–20 units, medium positive 21–80 units and high positive >80 units. All low positive samples were re-assayed and average measurements obtained were used for final classification.

B_2-glycoprotein I assays for the detection of β_2-glycoprotein I dependent aCL

Sera positive in the conventional anticardiolipin assays were assayed for IgA, IgG, and IgM β_2-glycoprotein I dependent aCL using commercially prepared Kits (INOVA Diagnostics, Inc. San Diego, California, USA). Samples were considered positive if antibody concentration was >20 standard units.

Statistical analysis

Statistical analysis was done using Stata 6.0 software (Stata Corporation, College Station, Texas, USA). The presence of aCL in any blood sample was initially determined in the whole group of 924 primiparae enrolled in the anticardiolipin antibody study. The second analyses were restricted to the 671 primiparae who gave two blood samples for anticardiolipin antibody testing. For these analyses, only subjects who were seropositive in two sera samples were classified as true positives. Pregnancy outcome was then ascertained in both aCL positive and aCL negative primiparae to determine if there was an association between aCL status and pregnancy outcome. Outcomes ascertained include term deliveries, pre-term deliveries, spontaneous abortions, stillbirths, low birth weight, pre eclampsia and eclampsia. Chi-square tests were used to compare frequencies of abnormal pregnancy outcomes between aCL positive and aCL negative primiparae. Using term deliveries as a reference category, the frequency of preterm deliveries and spontaneous abortions was compared between aCL positive and aCL negative primiparae. Analyses with preterm deliveries as the outcome excluded spontaneous abortions. The relative frequency of low birth weight, stillbirth, spontaneous abortions, pre eclampsia and eclampsia in aCL positive and aCL negative primiparae was also compared. Pregnancy outcomes were then ascertained both in the aspirin and placebo treatment groups. In these analyses, the effect of treatment with aspirin was examined by stratified analysis on assigned therapy to determine whether there was any modification of the association between aCL and pregnancy outcome.

The seroprevalence of aCL in normal pregnant women was estimated to be 2% (Harris and Spinnato 1991). We expected a 15% difference in aCL seroprevalence between primiparae with normal pregnancy outcome and those with abnormal pregnancy outcome. In order to obtain a stable estimate of aCL seroprevalence among primiparae allowing for a maximum error of 1% and assuming 17% aCL seroprevalence among women with abnormal pregnancy outcome a sample of 800 women was required. To demonstrate a 15% difference in aCL seroprevalence between women with abnormal pregnancy outcome at 95% level of significance and 80% power 75 subjects were required in each group. We over sampled to 924 women because of the possibility of dropouts and the need to obtain two blood samples from at least 800 women.

Assuming a 25% prevalence of spontaneous abortion among aCL positive women (Yasuda et al. 1995) a sample size of 335 such women would have been required to demonstrate a halving of risk with aspirin therapy at 95% level of significance and 80% power.

Results

Age on enrolment was ascertained in 901/924 (97.5%) of the participants. Their age ranged between 13–40 years. Age was not different with respect to aCL status ($p=0.53$) or assignment to aspirin or placebo ($p=0.08$). Similarly, there was no significant difference in age between primiparae who had term deliveries and those who had pre-term deliveries ($p=0.41$), abortion ($p=0.86$), stillbirth ($p=0.66$) or low birth weight ($p=0.41$). However, primiparae who had pre-eclampsia were slightly older 22.7 (4.7) years compared with those who did not have pre-eclampsia 21.5 (4.2) years ($p=0.04$). Sample characteristics are shown in Table I.

The prevalence of aCL was 32/671 or 4.8% (95CI 3.2–6.4) in primiparae who were tested on more than one occasion. aCL status in Jamaican primiparae is shown in Table II. A total of 49 of 924 primiparae or 5.3% (95%CI 3.9–6.7) were positive for aCL on at least one occasion. IgG was the most prevalent isotype observed in 31/49 (63.3%) of those seropositive in the first sample, 28/40 (70%) in the second sample and 23/32 (71.9%) of those who were positive in both sera samples (Table II).

Antibody titre in most of the seropositive primiparae was in the low positive range (10–20 units) in the first sample 33/49 (67.3%) and 26/40 (65%) in the second sample. Only 3/32 (9.4%) of those who were persistently positive were also positive for β_2-glycoprotein 1-dependent aCL. Being positive for β_2-glycoprotein 1-dependent aCL was not associated with abnormal pregnancy outcome.

Odds ratios and 95% confidence intervals for various pregnancy outcomes in the 671 primiparae who were tested on more than one occasion are shown in Table III. Given the reported prevalence of pre-eclampsia of 7.2%, at least two events would be expected in the aCL positive

Table I. Sample characteristics

Variable	*n*	(%)
Age (years) Mean (SD)	21.6 (4.3)	
Age range (years)	13–40	
Education level		
University	55	6.1
Secondary	540	59.9
Junior secondary	237	26.3
Primary	64	7.1
No formal education	1	0.1
Other	4	0.4
Total	901	100
Gravidity		
0	856	95.0
1	41	4.6
2	2	0.2
3	1	0.1
4	1	0.1
Total	901	100
Outcome of previous pregnancies		
Spontaneous abortion	24	53.3
Elective abortion	21	46.7
Total	45	100
VDRL		
Positive	10	1.2
Negative	814	98.8
Total	824	100

Table II. aCL status in primiparae

	Sample 1		Sample 2		Sample 1 & 2	
Isotype	*n*	(%)	*n*	(%)	*n*	(%)
aCL+ve	49	5.3	40	6.0	32	4.8
IgG	31	63.3	28	70	23	71.9
IgM	15	30.6	10	25	7	21.9
IgG and IgM	3	6.1	2	5	2	6.3
aCL−ve	875	94.7	631	94.0	639	95.2
Total	924	100	671	100	671	100
Antibody titre						
Low	33	67.3	26	65.0	19	59.4
Moderate	16	32.7	14	35.0	13	40.6
High	0		0		0	
Total	49	100	40	100	32	100

primiparae. The 0% prevalence detected makes it highly unlikely that aCL is strongly associated with pre-eclampsia in this population. The probability of our finding (0%) if the true prevalence were doubled to 14% in the presence of aCL would be 0.008. There were no differences in pregnancy outcome between aspirin and placebo treated primiparae with respect to aCL status. There was one stillbirth and two low birth weight deliveries among 28 aCL positive primiparae in the aspirin group. No adverse antenatal or perinatal events occurred in the placebo group ($n=20$). There was one pre-term delivery in each group.

Discussion

In the survey of aCL in Jamaican primiparae, the prevalence of aCL was 4.3% in women who were tested on more than one occasion. There were no statistically significant differences in pregnancy outcome between primiparae who were aCL positive and those who were aCL negative and there was no consistent pattern suggesting an increase in abnormal pregnancy outcome among those who were aCL positive. However, our study had inadequate power (16%) to demonstrate a difference in abnormal pregnancy outcome between aCL positive and aCL negative primiparae.

Treatment with aspirin had no effect on pregnancy outcome in aCL positive primiparae. Aspirin did not have any consistent beneficial effect in the trial from which these women were recruited (Golding 1998) and several other studies have not found benefit with aspirin in aCL positive women (Rai et al. 1997; Branch et al. 1992; Rai et al. 1995; Kuteh 1996). In light of these findings, the attempt to recruit the required number of aCL positive women to have adequate power to demonstrate whether aspirin had an effect in this subgroup was not warranted.

The aCL seroprevalence of 4.3% in women who were persistently positive was within the range observed in other studies (Harris and Spinnatto 1991; Lockwood et al. 1989; Parke et al. 1991). The prevalence of aCL in low risk pregnant women reported in various studies ranges between 2–7% (Harris and Spinnatto 1991; Lockwood et al. 1989; Parke et al. 1991). Most of aCL positive primiparae in this study had low antibody titres and only 3/32 (9.4%) women were positive for β_2-glycoprotein 1-dependent aCL the autoimmune type of aCL often associated with abnormal pregnancy outcome. These results suggest that aCL detected in this group were mainly of the non-autoimmune type which are not associated with thrombosis (McNeil et al. 1990; McNeil et al. 1991; Matsuura et al. 1990). The finding that abnormal pregnancy outcomes were infrequent in women who were aCL positive is also consistent with the results of other studies in which subjects had low aCL antibody titres (Harris and Spinnatto 1991; Lockwood et al. 1989; Parke et al. 1991).

Although it is agreed that aCL have a causative role in recurrent pregnancy loss (Gharavi 1989), there is still controversy over the kind of pregnancy loss associated with aCL. Some studies suggested that antiphospholipid antibody related pregnancy losses were mostly fetal deaths occurring in the second and third trimester (Geis and Branch 2001). Evidence from other studies suggested that most antiphospholipid antibody related pregnancy losses occur in the first trimester (Rai et al. 1997; Branch et al. 1992; Rai et al. 1991). If this is so, then the increase in spontaneous abortions may be noteworthy. In a randomised controlled trial of aspirin and heparin in pregnant women with recurrent miscarriages associated with antiphospholipid antibodies, 90% of all pregnancy losses occurred before the 13th week of pregnancy (Rai et al. 1997). Women who participated in this study, had been enrolled in the Jamaica Low Dose Aspirin Trial (Golding 1998) between 12–32 weeks' gestation by which time some antiphospholipid antibody related pregnancy losses may have already occurred (Geis and Branch 2001; Rai et al. 1997), and such women would not have been included in this study. In this study, treatment with aspirin appeared to have no effect on pregnancy outcome. While this finding is consistent with findings of some studies in which treatment with aspirin alone was not found to be effective in improving pregnancy outcome in women with aCL (Rai et al. 1997; Branch et al. 1992; Rai et al. 1995; Kuteh 1996), the small number of aCL positive cases could have contributed to the lack of effect observed in this study.

Table III. Odds ratios (95%CI) for various pregnancy outcomes in persistently aCL positive primiparae

	aCL+ve ($n=32$)		ACL−ve ($n=639$)		Total ($n=671$)			
Outcome variables	*n*	(%)	*n*	(%)	*n*	(%)	OR	95%CI
Antenatal complications								
Pre-eclampsia	0		44	6.9	44	6.6	–	
Eclampsia	0		3	0.5	3	0.5	–	
Perinatal outcomes								
Spontaneous abortion	1	3.1	5	0.8	6	0.9	4.08	0.48–36.0
Stillbirth	0		12	1.9	12	1.8	–	
Low birth weight	1	3.1	58	9.1	59	8.8	0.33	0.04–02.44
Gestation at delivery								
Term	28	87.5	585	91.5	813	91.4	0.55	0.15–1.8
Pre-term	2	8.3	27	4.2	29	4.3	1.55	0.35–6.64

Although it was not the objective of this study to establish that the subjects had the antiphospholipid syndrome, tests for both β_2-glycoprotein 1-dependent and β_2-glycoprotein 1-independent aCL were done on more than one occasion (Wilson et al. 1999). However, not all samples collected were tested for β_2-glycoprotein 1-dependent aCL. Only samples that were positive for aCL in the conventional anticardiolipin test were assayed for β_2-glycoprotein 1-dependent aCL. It is now well established that some samples can be positive for β_2-glycoprotein 1-dependent aCL, but negative for β_2-glycoprotein 1-independent aCL and vice-versa, due to the heterogeneity of antiphospholipid antibodies (Matsuura et al. 1992). Based on this, it is possible that the prevalence of β_2-glycoprotein 1-dependent aCL was underestimated in this study. While there is a possibility that the seroprevalence of β_2-glycoprotein 1-dependent aCL was underestimated, rates of adverse pregnancy outcomes would be expected to be higher than observed if there was significant underestimation. In this study, 1/6 (16.7%) of primiparae who had a spontaneous abortion were aCL positive compared with 53/138 (38.4%) in a study by Wharfe et al. (2004). The reasons for this difference are not obvious, but as shown in that study, the features of antiphospholipid syndrome did not appear to be different between aCL positive and aCL negative.

Conclusion

The prevalence of aCL in Jamaican primiparae was low, not associated with abnormal pregnancy outcomes and aspirin had no effect on pregnancy outcome. These findings suggest little or no risk of abnormal pregnancy outcome associated with aCL prevalent in Jamaican primiparae and support recommendations of previous authors (Harris and Spinnato 1991; Wharfe et al. 2004) against routine screening for aCL.

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