Anticardiolipin Status in Idiopathic Infertile Nigerian Females

Azenabor A[1*], Agha CU[2], Ogunmola IO[1], Bamiro SB[3], Osiagwu DD[1]

[1]*Department of Medical Laboratory Science, College of Medicine, University of Lagos*
[2]*School of Medical Lab Sciences, LUTH*
[3]*Department of Obstetrics and Gynaecology, College of Medicine, University of Lagos.*

***Corresponding Author:** **Azenabor A**, Department of Medical Laboratory Science, College of Medicine, University of Lagos*

1. INTRODUCTION

Idiopathic infertility refers to failure to conceive in a couple who have no definitive cause for infertility. The incidence of idiopathic infertility depends on the selection and age of the study population and to some extent on fertility evaluations [1]. This may account for the variations reported in different studies, though were all in the range of 10 – 20% [2,3,4]. Several theories have been tendered on the etiology of unexplained infertility. This includes diminished ovarian reserve [5], estradiol/progesterone ratio [6], impaired ovum pick up [7] and deregulation of the immune system [8]. Autoimmune processes and mechanisms are considered as contributing factors in some cases of unexplained infertility in both men and women, particularly when they are directed to antigens expressed in tissues and cells of the reproductive tract and genital secretion. Presence of antibodies to zona pellucida and endometrial antigens in women, are considered possible causes of infertility in a fraction of patients with unexplained infertility. Of particular importance are antiphospholipid antibodies, which include anticardiolipin among others [9]. Even though these antibodies have been found in individuals without any clinical consequences, approximately 15-20% of patients with these antibodies has been reported to have obstetric complications and up to 50-75% of these pregnancies was shown to exhibit poor outcomes [10]. In a more recent study, a high prevalence of IgG and IgM anti-cardiolipin antibodies were observed in women with unexplained infertility, unexplained recurrent abortion and intrauterine fetal death [11]. The mechanism of association of anticardiolipin antibodies with idiopathic infertility is complex and incompletely understood, though a previous study was able to provide a link between the incidences of anticardiolipin antibodies with polycystic ovarian syndrome – PCOS [12], while hyperprolactinaemia was postulated to affect fecundity not only by affecting ovulation and implantation through hormonal but also through immunological processes [13]. It is instructive to note that available reports on antiphospholipid antibodies in Nigeria females were on women with pregnancy complications, where a prevalence of 9.3% and 2.3% of lupus anticoagulant and anticardiolipin IgG antibodies respectively were observed [14]. Interestingly, among the five (5) cases of antiphospholipid antibody detected in females observed in Sagamu, Nigeria, pregnancy loss was the most frequent presentation, and anticardiolipin IgG was more commonly seen [15]. However, not much is known about the status of anticardiolipin antibodies in Nigerian females with unexplained infertility. The objective of this study includes the following:

1. Comparative assessment of the incidence of anticardiolipin antibodies (IgG and IgM) in idiopathic infertile Nigerian females and those with other known etiologies.

2. Comparison of the plasma levels of anticardiolipin antibodies (IgG and IgM) in idiopathic infertile Nigerian females and those with known etiologies.

3. Evaluation of a possible association between anticardiolipin antibodies and female reproductive hormones.

2. METHODS

This was a descriptive, cross sectional analytical study conducted on 176 women aged between 28 – 42 years attending the infertility clinic of the Lagos University Teaching Hospital (LUTH) Idi-Arabia,

Lagos. Inclusion criteria included women with a normal menstrual cycle ranging from 26–32 days, and not to have taken any contraceptive pills in the past one year. Exclusion criteria included women below 28 years of age, pregnant women, menopausal women, women with diabetes mellitus, thyroid disorders, connective tissue disease, autoimmune diseases, previous history of thrombo-embolism and women on immunosuppressive drugs or immune modulators. Ethical approval was obtained from the Health Research and Ethics Committee of the College of Medicine, University of Lagos, and Idi-Araba (Approval no - CM/HREC/MLS/05/16/006). Informed consent was obtained from the study subjects. Case report forms were administered in order to obtain information on biodata, histories pertaining to known illnesses, and menstrual cycle.

2.1. Laboratory Analyses

Fasting venous blood samples (5mls) were collected on the 14th day of a 28th day menstrual cycle from each subject for LH, FSH and anticardiolipin estimations and on the 23rd day of the cycle for prolactin, and progesterone. Anticardiolipin antibodies (IgG and IgM) and the reproductive hormones were estimated by enzyme linked immunosorbent (ELISA) method. The name of the kits used for IgG and IgM anticardiolipins was DiaMetra (Italy) while the Rapid lab kits (UK) was used for the reproductive hormones. The intra assay coefficient of variation for IgG anticardiolipin, IgM anticardiolipin, progesterone, prolactin , FSH and LH were 12.8%, 12.7%, 4%, 8.1, 2.9 and 5.9% respectively while the inter – assay coefficient of variation for IgG anticardiolipin, IgM anticardiolipin, progesterone, prolactin , FSH and LH were 11.8%. 11.5%, 9.3%, 9.7%, 3.2% and 9.0% respectively.

2.2. Diagnostic Criteria

1. Women with luteal phase progesterone level less than 18nmol/l despite normal levels of LH, FSH and prolactin were anovulatory

2. Women with prolactin levels above 550miu/l were classified as Hyperprolactinaemic despite normal levels of LH, FSH and progesterone.

3. Women with LH/FSH ratio greater than 3.0 were classified as having polycystic ovarian syndrome [16].

4. Women with normal hormonal profile results with their spouse already certified fertile during previous medical examination were classified as idiopathic or unexplained infertility.

5. Positive IgG and IgM anticardiolipins refer to serum levels $\geq$ 10Au/ml [17].

2.3. Statistical Analysis

The results generated from this study were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 17. Quantitative data was expressed as mean $\pm$ SEM. The difference between two means was assessed by student't' test. Within group and between group analyses was done using one way analysis of Variance (ANOVA). Post Hoc analysis was used to establish where significance lies in ANOVA. Categorical variables were assessed by Chi- square. Pearson's correlation coefficient was used to establish associations between groups. The level of statistical significance in all studies was defined as $p<0.05$.

3. RESULTS

The mean age (standard error of mean - SEM) and age range of the study subjects were 34(0.65) years and 22 – 42 years respectively. The number and proportions of females with idiopathic infertility were 64 (36.4%), anovulatory disorder 60 (34.1%), hyperprolactinaemia 26 (14.8%), and polycystic ovarian syndrome 26 (14.8%). While increased levels (mean $\pm$ SEM) of IgG anticardiolipin was observed in idiopathic infertility (8.01$\pm$3.05Au/ml) when compared with hyperprolactinaemia (7.88$\pm$ 0.49Au/ml), PCOS (6.07$\pm$0.36Au/ml) and anovulation (5.99$\pm$0.34Au/ml) (f = 7.89, p = 0.000); IgM anticardiolipin showed significantly higher levels in PCOS (10.14$\pm$0.51Au/ml) when compared with other groups (f = 4.60, p= 0.004). Incidence of IgG anticardiolipin antibodies were more prominent (65%) in idiopathic infertility, while IgM anticardiolipin recorded higher incidence (40.5%) among subjects with anovulatory disorders. An inverse correlation of IgG anticardiolipin was observed with prolactin in anovulatory disorder (r = -0.291, p = 0.024) and progesterone in idiopathic cases (r = 0.268, p = 0.032).

4. DISCUSSION

Autoimmune processes and mechanisms are implicated as one of the underlying causes of unsuccessful attempts for having a child, especially when all other reproductive hormones are normal. Unfortunately, infertile women usually display no signs or symptoms that reflect an autoimmune disease; hence unlike reproductive hormones, immunological parameters are not commonly sought for when investigating cases of infertility. This study was designed to assess the levels of anticardiolipin antibodies (IgG and IgM) in peripheral blood of Nigerian females with unexplained infertility compared with those of known etiologies and its possible associations with reproductive hormones.

In this study, hyperprolactinaemia was observed in 14.8% of the study subjects, while anovulatory disorder, polycystic ovarian syndrome and idiopathic causes were noticed in 34.1%, 14.8% and 36.4% respectively. Assessment of anticardiolipin status in these groups showed increased mean levels IgG and IgM anticardiolipin in subjects with unexplained infertility and polycystic ovarian syndrome respectively when compared with other known etiologies. The significant increase of IgG anticardiolipin levels may suggest the existence of a secondary immune response in most of the idiopathic infertile subjects. Thus, previous immunization with phospholipids, phospholipid – binding proteins or cross reactive antigen derived from infectious agents could be hypothesized. It is also worth mentioning that regardless of the etiology of infertility, more subjects show positivity (42%) for IgM anticardiolipin (≥ 10 Au/ml); however, among the IgG positive cases (22.7%), a greater percentage of them (65%) were those whose cause of infertility could not be traced to any hormonal imbalance as against 35.1% of IgM positivity rate of the same group.

Whilst IgM anticardiolipin positivity rates of 40.5%, 8.1%, 35.1% and 16.2% in anovulation, hyperprolactinaemia, unexplained infertility and polycystic ovarian syndrome respectively was observed, IgG anticardiolipin positivity rates were observed to be lower in the same groups, save for hyperprolactinaemic and idiopathic groups, where higher percentage of 20% and 65% respectively were documented. The high positive rate of IgM anticardiolipin observed in anovulatory disorder is consistent with that reported in other studies [18, 19]. This may be due to the ability of IgM antibodies to cause tiny blood clots in vessels preventing blood rich in hormones (LH and FSH) from reaching the ovaries and other reproductive organs leading to reduced follicular development and maturation [20, 21], which could also be another possible cause of anovulation in these subjects.

It is worthy of note that our reported incidence of IgG anti-cardiolipin antibodies in subjects with hyperprolactinaemia corroborates the reports of a previous study [22]. This could be attributed to the fact that prolactin enhances antibody production. Available evidence also suggests that hypophysectomy also suppresses the production of antibodies [23]. It is also instructive to note that prolactin is not only secreted by the anterior pituitary gland but also by extra-pituitary sites including the immune cells. Consequently, the elevated prolactin levels in these subjects, according to Bellis et al, could be due to coordinated bidirectional communications between prolactin and the immune system. This view was also supported by Abdul – Saud et al, who reported increased synthesis of interferon gamma and interleukin 2 by prolactin leading to production of antibodies especially those of the IgG type.

Pearson correlation coefficient was carried out to establish association of anticardiolipin status (IgG and IgM) with reproductive hormones in different infertile group studied. An inverse correlation of IgG anticardiolipin was observed with prolactin in anovulatory disorder (r = -0.291, p = 0.024) and progesterone in idiopathic causes (r = 0.268, p = 0.032). While IgM anticardiolipin show negative correlation with LH/FSH ratio in hyperprolactinaemic subjects (r = -0.268, p= 0.032), a positive correlation of IgM was shown with progesterone in polycystic ovarian syndrome (r = 0.675, p = 0.000). It can thus be inferred from the correlation studies that anticardiolipin status could influence reproductive hormones. This somewhat agrees with a previous study by Osamanagouglou et al [24] who reported an inverse relationship between progesterone and anticardiolipin antibodies in idiopathic infertile group.

Although not part of the objective of this study, a cursory assessment of the association of IgG anticardiolipins with IgM anticardiolipin antibodies showed that these antibodies were independent of each other (r = -0.022, p = 0.768). Presence of current infection reflected by IgM anticardiolipin status of infertile subjects is not influenced by previous IgG status. The mechanism involved may be due to the complex nature of autoimmune processes.

5. RESULTS

Table1. *Mean levels of anticardiolipin antibodies (IgM and IgG) in different etiologies of Infertility.*

Anticardiolipin antibodies	Anovulation n = 60	Hyperprolactinaemia n = 26	PCOS n = 26	Idiopathic Infertility n = 64	f values	p values
IgM	9.73 ±0.30	7.92 ±0.50	10.14 ± 0.51	9.64 ±0.30	4.60	0.004*
IgG	5.99 ±0.34	7.88 ±0.49	6.07 ±0.36	8.01 ±3.05	7.89	0.000*

*Values are presented as mean ±SEM. Statistical evaluation was performed using one way analysis of variance (ANOVA). *Significant. PCOS - Polycystic Ovarian Syndrome, IgM – Immunoglobulin M, IgG – Immunoglobulin G.*

Table2. *Post Hoc analysis of anticardiolipin antibodies (IgM and IgG) in Groups of Infertile Females studied*

Subjects	Groups	Mean difference	Standard error	p value
IgM				
Anovulation	Hyperprolactinaemia	1.09	0.56	0.002*
	Idiopathic	0.89	0.43	0.838
	PCOS	-0.42	0.56	0.462
Hyperprolactinaemia	Anovulation	-1.81	0.56	0.002*
	Idiopathic	-1.72	0.56	0.002*
	PCOS	-2.22	0.67	0.001*
Idiopathic	Anovulation	-0.09	0.43	0.838
	Hyperprolactinaemia	1.72	0.56	0.002*
	PCOS	-0.50	0.56	0.368
PCOS	Anovulation	0.42	0.56	0.462
	Hyperprolactinaemia	2.22	0.67	0.001*
	Idiopathic	0.50	0.56	0.368
IgG				
Anovulation	Hyperprolactinaemia	-1.89	0.63	0.003*
	Idiopathic	-2.01	0.48	0.000*
	PCOS	-0.08	0.63	0.899
Hyperprolactinaemia	Anovulation	1.89	0.63	0.003*
	Idiopathic	-0.12	0.62	0.844
	PCOS	1.81	0.74	0.015*
Idiopathic	Anovulation	2.01	0.48	0.000*
	Hyperprolactinaemia	0.12	0.62	0.844
	PCOS	1.93	0.62	0.002*
PCOS	Anovulation	0.08	0.63	0.899
	Hyperprolactinaemia	-1.81	0.74	0.015*
	Idiopathic	-1.93	0.62	0.002*

*significant at p<0.05

Table3. *Prevalence of subjects positive for IgG and IgM antibodies*

Anticardiolipin	Anovulation n (%)	Hyperprolactinaemia n (%)	Idiopathic n (%)	PCOS n (%)	f value	p value
IgM (>10Au/ml)	30(40.5)	6(8.1)	26(35.1)	12(16.2)	5.630	0.131
IgG (>10Au/ml)	4(10)	8(20)	26(65)	2(5)	24.790	0.000

PCOS – Polycystic ovarian syndrome, n =Number, % - percentage

Table4. *Pearson correlation Coefficient of anticardiolipin antibodies (IgM and IgG) with reproductive Hormones in Different Infertile Groups*

Infertile Subjects (n = 176)	Reproductive Hormones	IgM r (p)	IgG r (p)
Anovulatory Disorder (n =60)	LH	0.152 (0.246)	0.028 (0.833)
	FSH	0.198 (0.130)	0.238 (0.067)
	PRL	-0.239 (0.066)	-0.291 (0.024)*
	PROG	0.043 (0.742)	-0.066 (0.619
	LH/FSH	0.150 (0.919)	-0.169 (0.198)
Hyperprolactinaemia (n = 26)	LH	0.371 (0.062)	0.46 (0.824)
	FSH	0.057 (0.783)	0.193 (0.345)
	PRL	0.151 (0.461)	0.190 (0.352)
	PROG	-0.281 (0.165)	-0.242 (0.233)
	LH/FSH	-0.522 (0.006)*	0.196 (0.336)
Idiopathic (n = 64)	LH	-0.056 (0.660)	0.060 (0.637)
	FSH	-0.050 (0.692)	0.022 (0.862)
	PRL	0.177 (0.161)	0.018 (0.889)
	PROG	-0.136 (0.282)	-0.268 (0.032)*
	LH/FSH	-0.016 (0.897)	0.068 (0.591)
PCOS (n = 26)	LH	0.245 (0.228)	0.235 (0.247)
	FSH	0.246 (0.225)	0.193 (0.345)
	PRL	0.292 (0.148)	0.372 (0.061)
	PROG	0.675 (0.000)*	0.068 (0.740)
	LH/FSH	-0.032 (0.876)	0.068 (0.742)

Statistical evaluation was done using Pearson correlation coefficient. A value of p less than 0.05 was accepted as significant.

6. CONCLUSION

From the empirical data generated, the IgG and IgM status of anticardiolipin antibodies were significantly higher in infertile subjects with unexplained causes and polycystic ovarian syndrome respectively. A higher prevalence of anticardiolipin (IgG) was observed in infertile subjects with unexplained causes. Anticardiolipin is significantly associated with some of the female reproductive hormones

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