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Relation between anti-phospholipid antibodies and failed intra-cytoplasmic sperm injection

Abstract

Objectives: This study designed to detect Relation between anti-phospholipid antibodies (APA) and failed intra-cytoplasmic sperm injection (ICSI).

Patients and methods: One hundred women with failed one or more ICSI trials were included in this cross sectional study. Women included in this study were less than 35years old and had normal Follicle stimulating hormone (FSH), Lutenizing hormone (LH), Estradiol (E2), Thyroid-stimulating hormone (TSH) and prolactin. Blood samples collected from studied women with failed ICSI trials for screening of anti-cardiolipin (ACL) antibodies (IgG & IgM) and lupus anticoagulant (LA) at any time of menstrual cycle and repeated 6 weeks later to confirm the diagnosis. Primary outcome measures; relation between anti-phospholipid antibodies (APA) and failed ICSI.

Results: ACL IgM detected in 20cases of studied women with failed ICSI (20%), while the ACL IgG detected in 18cases of failed ICSI (18%) and LA detected in 19cases of failed ICSI (19%). The total number of failed ICSI that had ACL and LA was 23 patients (23%). In Positive cases, the mean ACL IgM significantly increased from 24.5±1.9 at the start of the study to 27.5±0.9MPL U/ml 6weeks after (p=0.002; 95% CI; -3.8, -3, -2.1). While the mean ACL IgG was 28.05±2.094 at the start of the study and decreased to 26.94±5.45GPL U/ml 6weeks after (non-significant difference). Mean ACL (IgM & IgG) increased with increased number of failed ICSI trials (positive relation). In addition, there was positive correlation between LA and number of failed ICSI trials.

Conclusion: APA could adversely affect the implantation and could compromise the success of IVF treatment.

Keywords: anti-phospholipid antibodies, intracytoplasmic sperm injection

Volume 1 Issue 2 - 2015

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Received: November 23, 2015 | **Published:** December 07, 2015

Abbreviations: ICSI, intra-cytoplasmic sperm injection; FSH, follicle stimulating hormone; LH, lutenizing hormone; E2, estradiol; TSH, thyroid-stimulating hormone; ACL, anti-cardiolipin; LA, lupus anticoagulant; APA, anti-phospholipid antibodies; ART, assisted reproductive technique; PID, pelvic inflammatory disease; DRVVT, dilute Russell's viper venom test

Introduction

Intra-cytoplasmic sperm injection (ICSI) is one of the modalities of assisted reproductive technique (ART), which treats couples in whom the male partners has azoospermia or oligospermia. ICSI indicated for treatment of anti-sperm antibodies, low sperm motility or abnormal sperm morphology.[1] Failure of conception after transfer of good embryos is a significant clinical problem.[2–5] Increased levels of autoantibodies initially described in patients with endometriosis and recurrent spontaneous abortion.[6] Autoantibodies detected in infertility screening, include Anti-cardiolipin (ACL) antibodies, Anti-phospholipid antibodies (APA) and anti-thyroid antibodies.[7,8]

Di Simone et al.,[9] demonstrated that the binding of APA to human trophoblast affecting trophoblast invasiveness and cytotrophoblast differentiation, leading to failure of blastocyst implantation.[9] This study designed to detect the relation between anti-phospholipid antibodies and failed ICSI.

Patients and methods

One hundred women with failed one or more ICSI trials were included in this study, after informed consent and approval of the study protocol by the institute ethics committee. Failed ICSI trials means negative pregnancy test after transfer of good embryos (regular blastomeres & no minor fragments). Women included in this study were less than 35years old and had normal Follicle stimulating hormone (FSH), Lutenizing hormone (LH), Estradiol (E2), Thyroid-stimulating hormone (TSH) and prolactin. Diabetic women or women receiving hormonal treatment or women with gross uterine abnormalities excluded from this study. Women with history of pelvic inflammatory disease (PID) or grade III or IV endometriosis also excluded from this study.[10]

Blood samples collected from studied women with failed ICSI trials for screening of anti-cardiolipin (ACL) antibodies (IgG & IgM) and lupus anticoagulant (LA) at any time of menstrual cycle and repeated 6weeks later to confirm the diagnosis.[11] Anti-cardiolipin IgG & IgM: The Autostat II assay for detection of autoantibodies is a solid phase immunosorbent assay (ELISA) indicated by a color reaction of an enzyme and substrate. The Autostat II wells coated with purified antigen. Highly purified cardiolipin is bound to micro wells saturated with β2 glycoprotein I. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Horseradish peroxidase conjugate anti-human IgG & IgM immunologically detect

the bound patient antibodies forming a conjugate/antibody/antigen complex. The test uses standards that are traceable to the Harris et al.,[11] reference sera. The concentration of ACL measured in international units and the results reported in GPL or MPL Units per ml.[12]

Test considered positive when ACL IgG>23GPL U/ml and/or IgM>11MPL U/ml, have established in blood on two or more occasions at least 6weeks apart. Lupus anticoagulant (LA): LA I Screening reagent and LA II confirmation reagent are simplified Dilute Russell's Viper Venom Test (DRVVT) reagents for detection of lupus anticoagulant in one stage clotting tests. LA I screening reagent: Simplified DRVVT reagent to screen for the presence of LA. LA II screening reagent: Phospholipids rich RDVVT reagent for the specific correction LA. If the LA I screening reagent-clotting time was within the normal range, no further testing for LA needed. If LA I screening reagent-clotting time was>2 standard deviation longer than the mean of normal plasma (normal≥ 20), the result considered abnormal and investigated further. The result expressed as a ratio of the clotting times of LA I screening reagent divided by LA II confirmation reagent.

a. If ratio is>2.0=LA is strongly present.

b. If ratio is 1.5-2.0=LA is moderately present.

c. If ratio is 1.2-1.5=LA is weakly present.

Justification and statistical analysis

Using data from previous studies and EpiInfo® version 6.0, a sample size of ≥90 women needed to produce a significant difference. Statistical analysis was done using SPSS (Statistical Package for Social Sciences); computer software version 18 (Chicago, IL, USA). Mean and SD (standard deviation) were used to represent numerical variables, while, number and percentage were used to represent categorical variables. Student's t was used for analysis of quantitative data, Chi-square (x^2) test for analysis of qualitative data. P value<0.05 was considered significant.

Results

Eighty-six of the studied women had single failed ICSI trial, 9 women had two failed ICSI trials and 5 women had 3 failed trials. ACL IgM detected in 20 women of failed ICSI (20%), while the ACL IgG detected in 18 women of failed ICSI (18%) and LA detected in 19 women of failed ICSI (19%). The total number of studied women with failed ICSI who had ACL and LA was 23 women (23%). In Positive cases, the mean ACL IgM significantly increased from 24.5±1.9 at the start of the study to 27.5±0.9MPL U/ml 6weeks after (p=0.002; 95% CI; -3.8, -3, -2.1). While the mean ACL IgG was 28.05±2.094 at the start of the study and decreased to 26.94±5.45GPL U/ml 6weeks after (non-significant difference; p=1; 95% CI; -1.5, 1.1, 3.75) (Table 1).

Mean ACL (IgM & IgG) increased with increased number of failed ICSI trials (positive relation). In addition, there was positive correlation between LA and number of failed ICSI trials; LA detected in 10.46% of women with failed single ICSI trial, in 55.5% of women with failed two trials and in 100% of women with failed three trials (LA increased with increased the numbers of failed ICSI trials (Table 2).

Table 1 Mean ACL (IgM & IgG) at start of the study and 6weeks after

Variables	Mean±SD	P value (95% CI), Significance
ACL IgM (MPL U/ml) At the start of the study	24.5±1.9	
ACL IgM (MPL U/ml) After 6 weeks	27.5±0.9	0.002 (-3.8,-3,-2.1), Significant
ACL IgG (GPL U/ml) At the start of the study	28.05±2.094	
ACL IgG (GPL U/ml) After 6 weeks	26.94±5.45	1 (-1.5,1.1, 3.75), Significant

ACL, anti-cardiolipin antibodies; CI, confidence interval; Statistical analysis done using student's T test

Table 2 The Relation between ACL (IgM & IgG) + LA and the Number of Failed ICSI Trials

Variables	One trial of ICSI (Number = 86)	Two trials of ICSI (Number = 9)	Three trials of ICSI (Number = 5)	P value (95% CI), Significance
ACL IgM (MPL U/ml)				P1=0.001(-17.4,-15.7,-13.9), Significant* P2=0.0001(-16.9,-15.3,-13.8), Significant*
Mean±SD	5.81±5.84	21.55±1.9	21.2±1.4	P3 = 0.2 (-1.3,0.3, 2.0), Non-significant*

Citation: Awadalla AM. Relation between anti-phospholipid antibodies and failed intra-cytoplasmic sperm injection. *MOJ Womens Health.* 2015;1(2):49–52. DOI: 10.15406/mojwh.2015.01.00011

Table Continued..

Variables	One trial of ICSI (Number = 86)	Two trials of ICSI (Number = 9)	Three trials of ICSI (Number = 5)	P value (95% CI), Significance
ACL IgG (GPL U/ml) Mean±SD	6.05±6.31	26.33±2.1	28.4±1.9	P1=0.001(-22.1,-20.3,-18.36), Significant* P2 = 0.01(-24.4,-223,-20.2), Significant* P3=0.1(-4.2,-2.07,0.08), Non-Significant*
Positive LA Number (%)	9 (10.46%)	5 (55.55%)	5 (100%)	P 1 = 0.01, Significant** P2 = 0.002, Significant** P3 = 0.4, Non-Significant**

* Statistical analysis done using student's T Test, ** Statistical analysis done using chi-square test (X^2)

ACL: Anticardiolipin Antibodies; CI: Confidence Interval; ICSI: Intracytoplasmic Sperm Injection; LA: Lupus Anticoagulant

P1 = p value when women with failed One ICSI Trial Compared with Women with Failed two Trials.

P2 = p value when women with failed One ICSI Trial Compared with Women with Failed Three Trials.

P3 = p value when failed two ICSI Trials Compared with Women with Failed Three Trials.

Discussion

ICSI, is the standard treatment for couples in whom the male partners has azoospermia or oligospermia, however, a debate is continued concerning other indications of ICSI particularly when the male has normal semen parameters.[13] Eighty-six of the studied women had single failed ICSI trial, 9 women had two failed ICSI trials and 5 women had 3 failed trials. In this study, The ACL IgM detected in 20 women of failed ICSI (20%), while the ACL IgG detected in 18 women of failed ICSI (18%) and LA detected in 19 women of failed ICSI (19%). The total number of studied women with failed ICSI who had ACL and LA was 23 women (23%), Chilcott et al.,[14] found that about 23% (89/380) of patients referred for IVF were positive for ACL or LA, also, Ghazeeri et al.,[15] concluded that 20-25% of all women undergoing IVF have positive APA compared to controls (5%).[15]

Elena et al.,[11] found that the presence of APA, was the second frequent abnormality among IVF failure patients (19%), after anti-thyroid antibodies, they believed that APA bind to human trophoblast in a dose dependent manner and adversely affect trophoblast invasiveness as well as differentiation of cytothrophoblast into a syncytiothrophoblast.[11] McIntyrea John and colleagues suggested that the presence of APA may induce blastocyst implantation failure.[16] In this study, there was a significant positive relation between ACL (IgM & IgG) and the number of failed ICSI trials, the mean value of the ACL (IgM & IgG) increased with increased number of failed ICSI trials.

Zdenka et al.,[17] found that sera from patients after two and more IVF procedures, are immunologically more active than sera from women after one unsuccessful IVF.[17] Grandone et al.,[18] concluded the high prevalence of APA was detected in patients with at least three unsuccessful IVF attempts. Grandone et al.,[18] suggested that repeated IVF failure and high levels of APA in some patients could be explained by direct influence of the APA on phospholipids of blood vessels network in the uterine mucosa, or on the surface of embryos, which could affect the implantation. This study concluded that APA could adversely affect the implantation and could compromise the success of ICSI trials. Large comparative study needed to prove definite relation between APA and repeated ICSI failures.

Acknowledgements

I would like to express my appreciation and acknowledgment to professor doctor Ibrahim A Abdelazim, for his effort and support during conduction of this study.

Conflict of interest

The author declares no conflict of interest.

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Citation: Awadalla AM. Relation between anti-phospholipid antibodies and failed intra-cytoplasmic sperm injection. *MOJ Womens Health.* 2015;1(2):49–52. DOI: 10.15406/mojwh.2015.01.00011