THE PREVALENCE OF ANTIPHOSPHOLIPID ANTIBODY IN WOMEN WITH RECURRENT PREGNANCY LOSSES AND IN INFERTILE WOMEN WITH MULTIPLE IMPLANTATION FAILURES AFTER IN-VITRO FERTILIZATION

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Abstract
This study was designed to investigate the prevalence of anticardiolipin (aCL), antiphosphatidylserine (aPS) and β2-glycoprotein I (β2-GPI) antibodies in women with repeated spontaneous abortion (RSA) and repeated in-vitro fertilization-embryo transfer failure (IVF-ET-F). The study included 45 with three or more RSA of unknown etiology with the same partner (RSA group), 40 women with IVF-ET failures after two or more IVF cycles (IVF-ET-F group) and 10 normal multiparous healthy non-pregnant women with documented uncomplicated pregnancies (Control group). After full history taking and complete general and obstetric examination all patient gave blood samples for determination of aPS, aCL and β2-GPI IgG and IgM antibodies by ELISA. Anticardiolipin antibodies was detected in 12 patients with RSA (26.7%) and 11 patients (27.5%) with IVF failure, while aPS antibodies were detected in 6 (13.3%) and 14 (35%) patients in both groups respectively. There was a significant increase in the number of patients with positive aCL and aPS antibodies in both groups, compared to control group. Intergroup comparison showed increased number of IVF failure patients having antibodies that was non-significant in aCL but was significant in aPS. Anticardiolipin IgG antibodies were detected in 11.1% and IgM in 13.4% in patients with RSA, whereas in IVF-ET-F group, IgG antibodies were detected in 17.5%, IgM antibodies in 7.5%. In patients with RSA IgG-aPS antibodies were detected in 4.4%, IgM antibodies in 8.9%, while in patients with IVF failure, IgM-aPS antibodies were detected in 10% and IgG antibodies in 20% of patients with
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an increased number of IgG positive patients in IVF-ET-F group compared to those with RSA, and the difference was significant in case of IgG-aPS. Anti-β2-glycoprotein I antibodies could not be detected in controls, moreover, no patient had both IgG and IgM in either group, only 4 patients in each group had IgG antibodies and only one patient in IVF-ET-F group had IgM antibodies. It could be concluded that repeated pregnancy loss or IVF embryo transfer failure may be an entity of antiphospholipid syndrome associated with high frequency of IgG anticardiolipin and antiphosphatidylserine antibodies and such patients must be investigated for the presence and titre of these antibodies.

Introduction

Approximately 10-15% of couples desiring children suffer from infertility. However, even after a thorough evaluation, the cause of their inability to conceive remains unknown in at least 10% of the cases. Despite treatment, including IVF, many of these couples remain childless. The search for answers to unexplained infertility has concentrated on the possible role of immunology in reproductive failure. (Ghazeeri & Kutteh, 2001).

Recurrent spontaneous abortion is a common complication of pregnancy that may affect as many as 2% of women in reproductive age. (Coulam, 1991). Although genetic, anatomic and hormonal causes have been implicated in the etiology of RSA, (Carp et al., 1990), >60% of cases remain unexplained. (Kwak-Kim et al., 2003). Various immunological abnormalities have been reported in women with RSA of unknown etiologies including autoimmune abnormalities such as positive antiphospholipid antibodies, antinuclear antibodies, anti-thyroglobulin antibodies and antimitochondrial antibodies, and increased cellular immunity such as elevated natural killer cell levels and its cytotoxicity. (Ruiz et al., 1996).

Interestingly, these immunological abnormalities also occur in infertile women who have implantation failures after multiple IVF cycles. (Coulam et al., 1997). Women undergoing assisted reproductive procedures, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), fail to achieve pregnancy in approximately 70% of cases. (Martinielli et al., 2003). Kikuchi et al., (2003), investigated the impact of antinuclear antibody (ANA) on pregnancy rates after IVF embryo transfer (IVF-ET) and their findings suggested that ANA might have an impact on implantation failure in women treated by IVF-ET or ICSI-ET and concluded that ANA reduced the pregnancy rates in the first IVF-ET or ICSI-ET cycles but not the cumulative pregnancy rates. (Martinielli et al., 2003). The antiphospholipid antibody family includes a heterogeneous population of autoantibodies whose specificity is directed against phospholipids and their complex with plasma proteins. It is recognized that the presence of IgG and IgM anticardiolipin antibodies and lupus anticoagulant. (Wilson et al., 1999). It has also been demonstrated that these antibodies are directed to plasma proteins bound to anionic phos-
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Furthermore, thrombosis in antiphospholipid syndrome may be another mechanism for impending success of IVF embryo transfer and/or induce pregnancy loss. However, the mechanism of such thrombosis is still unknown, but a number of hypotheses have been proposed to explain the mechanisms by which antiphospholipid antibodies may promote thrombotic events. These include the activation of endothelial cells, oxidant-mediated vascular endothelium lesions, interference with or modulation of the phospholipid-binding proteins regulating hemostasis, and mechanisms similar to heparin-induced thrombocytopenia. (Fiedler & Wurfl, 2004).

Reduced fibrinolytic activity has also been described in patients with antiphospholipid syndrome, and may be responsible for thrombotic events and antibodies directed against tissue-type plasminogen activator might lead to a hypofibrinolytic state. (Palomo et al., 2004).

This study was designed to investigate the prevalence of anticalcdilipin (aCL), antiphosphatidylserine (aPS) and β2-glycoprotein I (β2-GPI) antibodies in women with repeated spontaneous abortion (RSA) and repeated in-vitro fertilization-embryo transfer failure (IVF-ET-F).

Materials & Methods

Patients

The study design was a prospective controlled study and was conducted at Obstetrics and Gynecology Department at Benha University Hospital in conjunction with Medical Biochemistry Department, Benha Faculty of Medicine, Zagazig University.

Inclusion criteria for this study were: (i) fertile women with three or more RSA of unknown etiology or (ii) infertile women with two or more IVF-ET failures, who had two or more embryos transferred per each IVF cycle, excluding donor oocyte cycles; (iii) not pregnant; (iv) no or no more than one live born infant; (v) age ranges 25-45 years old; and (vi) no active disease including autoimmune disease. All patients underwent physical examination, past history review and review of system and blood tests including complete blood count and thyroid function tests. Blood tests were performed in the Benha University hospital laboratory.

The recurrent spontaneous abortion group (RSA group) included 45 women with three or more RSA of unknown etiology with the same partner. Three had one child. All had pregnancy losses during the first trimester of gestation. None had active or a history of autoimmune disease with no apparent causes of recurrent abortion such as chromosomal, endocrine, anatomical, or infectious etiologies were documented for previous pregnancy losses. None had infertility or received IVF cycles, (Table 1). A total of 40 women with IVF-ET failures after two or more IVF cycles comprised IVF-ET failure group (IVF-ET-F group). No woman in this group had active or a history of autoimmune disease with no apparent...
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**Sampling:**
All the study and control subjects gave blood samples prior to any treatment including IVF cycles. No one was on any medication. Blood was allowed to clot, centrifuged and serum was collected and stored at -80°C until assayed for IgG and IgM antiphospholipid antibodies.

**Methods:**
All serum samples were evaluated for IgG and IgM antibodies against cardiolipin (ACL antibodies) (Harris et al., 1994), phosphatidylserine (APS antibodies), phosphatidylcholine (PC antibodies) and phosphatidylethanolamine (PE antibodies).