

Research Paper: Protein C Activity in Women With Unexplained Infertility



Mohamed Samir Sweed^{1*}, Ihab Fouad Serag-Eldin¹, Islam Tharwat Wafa¹

1. Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.



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ABSTRACT

Objectives: The relation between thrombophilia and infertility has drawn the attention in last few years. Most studies investigating the effect of inherited thrombophilia on infertility studied the relationship between thrombophilia and implantation failure. It was found to be a possible cause of impaired implantation in some subgroups of infertile women. Still thrombophilia screening in general infertile population is unjustified and in fact may lead to increased patient anxiety after a positive test result together with the increased cost. Due to the poor information available concerning the relationship between inherited thrombophilia and infertility, we performed a case-control study in order to evaluate the prevalence of protein C activity in women with unexplained infertility.

Materials & Methods: This is a case-control study conducted to evaluate the prevalence of protein C activity in women with unexplained infertility conducted at Ain-Shams University Maternity Hospital. 48 women with unexplained primary infertility and another 48 fertile women of matched age group as their control were recruited from July 2011 to January 2013. Using the BIOPHEN Protein C 2.5 assay, protein C was measured following a specific activation with Protac, an enzyme extracted from snake venom. Activated protein C (APC) then specifically cleaves the specific substrate SaPC-21, releasing para-Nitroaniline (pNA), and its color is measured at 405 nm. 2(4.2%) cases of protein C deficiency were encountered in the unexplained infertility group while none found in the control group.

Results: This study failed to find relationship between protein C activity and unexplained infertility compared to normal fertile population. In spite of finding 2 cases with protein C deficiency in the unexplained infertility sample population and none in the control group, this difference failed to reach significance (P=0.495 and P=0.076 respectively).

Conclusion: In conclusion, this study failed to find any association between protein C activity and unexplained infertility.

* Corresponding Author:

Mohamed Samir Sweed, MD

Address: Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Tel: +2 (10) 01222047

E-mail: drmsweed@med.asu.edu.eg

1. Introduction

Infertility is defined as failure of conception for one year with frequent, unprotected coitus [1]. It has multiple etiologies, but approximately 15-17% of cases have no apparent etiology and are defined as unexplained infertility [2]. Unexplained infertility is suggested when standard investigations as tests of ovulation, tubal patency and semen analysis are normal [3]. The value of other tests than these basic tests in the management of infertility are still not proven [4].

The relation between thrombophilia and infertility has drawn the attention of some researchers in last few years [5, 6]. Most studies investigating the effect of inherited thrombophilia on infertility studied the relationship between thrombophilia and implantation failure [7, 8]. It was found to be a possible cause of impaired implantation in some subgroups of infertile women [9]. The majority of thrombophilias' related placental vascular complications during pregnancy have been attributed to the protein C pathway [10]. Also, the majority of the increased hypercoagulable states encountered in women who conceived after assisted reproduction were found to be related to a decline in protein C levels and an increase in the acquired Activated Protein C (APC) resistance [11].

Still thrombophilia screening in general infertile population is unjustified and in fact may lead to increased patient anxiety after a positive test result together with the increased cost [12]. Due to the poor information available concerning the relationship between inherited thrombophilia and infertility, we performed a case-control study in order to evaluate the prevalence of protein C activity in women with unexplained infertility.

2. Materials and Methods

Study design

This case-control study was conducted at Ain-Shams University Maternity Hospital from July 2011 to January 2013. 48 women with unexplained primary infertility were recruited from the infertility outpatient clinic; another 48 fertile women who got pregnant at least once in the previous year were recruited from outpatient gynecology clinic as control. The study was approved by the ethical committee of the Department of Obstetrics and Gynecology, Ain Shams University and informed written consents were obtained from all participants before commencement of the study.

Women with unexplained infertility were diagnosed when; normal husband's semen analysis according to WHO criteria 2010 [13], documented ovulation diagnosed either by mid-luteal serum progesterone ≥ 10 ng/ml or transvaginal ultrasound folliculometry, normal FSH, LH, TSH and serum prolactin levels, patent fallopian tubes as evident either by hysterosalpingogram (HSG) and/or laparoscopy were all encountered. Women aged less than 18 years or more than 35 years, with secondary infertility, anovulatory cycles, any uterine pathology or medical disorder that might affect pregnancy (e.g. diabetes or thyroid dysfunction) were excluded from the study.

Assay principle

BIOPHEN Protein C 2.5 kit is a chromogenic assay for measuring Protein C activity in human citrated plasma. Protein C is a vitamin K dependent human protein, which inhibits and regulates coagulation through specific cleavages of Factors Va and VIIIa, suppressing their procoagulant cofactor activity [14]. Using the BIOPHEN Protein C 2.5 assay, Protein C was measured following a specific activation with Protac, an enzyme extracted from snake venom (Agkistrodom C Contortrix) [15]. Activated Protein C (APC) then specifically cleaves the specific substrate SaPC-21, releasing para-Nitroaniline (pNA), and its color is measured at 405 nm. There is a direct relationship between color development and Protein C activity in the tested plasma.

Sample size justification and statistical analysis

Sample size was calculated using Epi Info® version 6.0, setting the power (β) at 80% and the significance level (α) at 0.05. Data from a previous study indicated decreased levels of activated protein C resistance rate by 25% in women with infertility [16]. Calculations according to this value produce a minimal sample size of 48 women in each group.

Statistical analysis was done using Stata© version 11 (StataCorp LP, College Station, TX, USA) and MedCalc© version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium). The D'Agostino Pearson test was performed to test the normality of numerical data distribution. The D'Agostino Pearson test was performed to test the normality of numerical data distribution. Normally distributed numerical data are presented as mean and SD. Qualitative data are presented as number and percentage. For normally distributed numerical data, the independent-samples (unpaired) Student t test was used to compare the difference in the means between the two study groups. The Pearson chi square test was used for comparison of the two groups as regards differences in categorical data. Fisher's exact test was used in place of the chi square test if

Table 1. Comparison of protein c activity and incidence of protein c deficiency among the two groups

| Variable | Group I (n=48) | Group II (n=48) | P |
|----------------------------------|----------------|-----------------|-------|
| Protein C activity (% of normal) | 97.99(20.17) | 104.99(18.01) | 0.076 |
| Protein C deficiency | 2(4.2%) | 0(0.00) | 0.495 |

Data are presented as number (%).

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>20% of the cells in any contingency table had an expected count of <5. P<0.05 was considered statistically significant.

3. Results

A total of 48 women unexplained infertility were included as group I. A second set of 48 fertile control women were included as group II. There was no statistically significant difference between women in both groups regarding age or body mass index, 29.04 years (SD=3.32) and 26.01 kg/m² (SD=3.35) in group I respectively, and 29.77 years (SD=3.89) and 26.23 kg/m² (SD=2.54) in group II respectively. There was also no significant difference between the two groups regarding protein C activity or incidence of protein C deficiency (Table 1).

4. Discussion

The role of thrombophilia in unexplained infertility and implantation failure is controversial and poorly understood [9]. Many studies addressed the relation of hereditary thrombophilia and infertility especially cases related to assisted reproduction and repeated IVF failure. Reduced protein C global assay level was found in infertile women prior to assisted reproduction treatment, a finding suggesting unique anticoagulation protein C pathway in infertile compared to fertile women [17]. It has been established that the majority of placental vascular complications associated with thrombophilias during pregnancy have been related to the protein C pathway [10, 18].

To our knowledge this is the first study to address specifically the relation between protein C activity and unexplained infertility (PubMed search engine, January 1990 to January 2015, search terms included performed in the English language were “Protein C” “Protein C activity”, “infertility” and “unexplained infertility” in different combinations).

This study failed to find relationship between protein C activity and unexplained infertility compared to normal fertile population. In spite of finding 2 cases with protein C deficiency in the unexplained infertility sample population and none in the control group, this difference failed to reach significance. This result is consistent with Fatini et al. 2012 who studied the relationship between inherited thrombophilia and

different causes of infertility, where 6 patients (2.6%) with unexplained infertility had protein C and s deficiency in comparison to 3 fertile women (1.2%) and the p value was 0.6 which was insignificant [19]. Another study found the incidence of protein C deficiency even more common in fertile women rather than women with unexplained infertility, yet; still the difference was statistically insignificant [9].

A study including Iranian infertile women found decreased levels of activated protein C resistance in 25% of infertile patients [16]. Another case-control study evaluated protein C pathway using (HemosIL ThromboPath) assay found thrombophilia ascertained in 13% of women with infertility. They concluded this assay to be an efficient assay for screening women with pregnancy complications and might provide prognostic information during pregnancy progression [20].

The majority of literature addressed the relation of thrombophilia and IVF failure rather than infertility itself, still results are contradicting and needs more evidence and research. While some studies suggest the association between recurrent IVF failure and increased incidence of thrombophilia [7-9, 21, 22] and even in particularly in the subgroup with unexplained fertility [21], others suggested that screening for thrombophilia is not indicated in cases with unexplained reproductive failure [23-26].

5. Conclusion

In conclusion, this study failed to find any association between protein C activity and unexplained infertility. Still this pilot study included limited number of participants and didn't investigate other thrombophilia markers to assess their relation with unexplained infertility, especially that others have demonstrated the relationship between other inherited thrombophilia markers and unexplained infertility [19].

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Conflict of Interest

The authors declared no conflicts of interest.

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