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Association of Congenital Thrombophilia in Unexplained Infertility: By Chance or By Cause

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Abstract

Objective: To find incidence of congenital thrombophilia in unexplained infertility versus fertile female population with intend to determine if thrombophilia is a possible cause and which type of thrombophilia is more prevalent in unexplained infertility.

Methods: Study group (Group A) comprised of 40 infertile patients of age between 20-40 years in whom no cause of infertility was identified. The control group comprised of 40 healthy women with proven fertility, matched for age, with no history of thrombo-embolic events. Both the groups were tested for inherited thrombophilia included Protein C, Protein S, Anti-thrombin III, Activated Protein C Resistance, Homocysteine, factor V Leiden (PCR), Prothrombin G20210 G gene and M THFR (677c/T)gene mutation.

Results: Overall 14 (35%) subjects in the unexplained infertility group, and 5 (12.5%) in the control group were detected with positive thrombophilic factor (p value-0.023). Out of 14 positive cases, 7/14(50%) had more than one factor (35% had 2 and 15% had 3 factors)thus 23 thrombophilia factors in 14 women in group 1 in comparision of onlt 7 in 5 in (2 with 2 factors)group2 (p=0.005). Out of 23, Protein S (10%) and Antithrombin III (10%) had the highest frequency followed by Homocysteine (7.5%), Protein C (7.5%), MTHFR gene mutation (7.5%), prothrombin 20210 (5%), Factor V Leiden (5%) and APCR (5%)in group1 Out of the 7 thrombophilia factors in controls, Factor V Leiden mutation and APCR had the highest frequency (5%) followed by Protein S, Protein C, and Homocysteine (2.5%). APCR and Factor V leiden were similar in both groups whereas Hyperhomocysteinaemia combined with MTHFR gene mutation was more in unexplained infertility andprothrombin gene was found only in unexplained infertility

Conclusion: There is significant association of thrombophilia with unexplained infertility Hyperhomocystinaemia combined with MTHFR gene mutation and APCR with prothrombin gene mutation are more likely to be associated with unexplained infertility. Thrombophilia evaluation may be included along with other tests in evaluation of unexplained infertility.

Keywords: Unexplained Infertility, Congenital Thrombophilia.

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INTRODUCTION

Infertility affects between 15 to 20% couples of reproductive age and is a major health issue. Despite the best available assisted reproduction techniques there is a large no of patients who are not able to conceive. Nearly 1/3rd of patients of infertility are unexplained infertility, in which no abnormality is revealed and treatment is only empirical which may include expectant observation, ovulation induction, intrauterine insemination or even in vitro fertilization.[1]

Undiagnosed early pregnancy loss or biochemical pregnancy loss may also be a cause of infertility. The majority of studies conducted to evaluate failure to conceive in assisted reproduction have focused on the problems that occur following laboratory fertilization i.e. implantation of the embryo in the woman's uterus.[2] Factors that need to be taken into consideration include improving endometrial receptivity and identifying intervening factors associated with immunological response and the genetic characteristics of the woman, including her potential for thrombosis during pregnancy and implantation of the embryo.[3] Recently, a hypothesis has been raised that the same factors of thrombosis associated with the occurrence of recurrent pregnancy loss may also affect the early phase of the embryo implantation process and leading to infertility[2], however, there is no consensus in the literature on this subject. The haematological changes that lead to hypercoagulability, with a consequent increase in the occurrence of thrombosis, have been cited as factors that hamper the process of embryo implantation.[2,4,5] Hence thrombophilia should be considered an adverse factor in cases of embryo implantation failure which may present as unexplained infertility. Thrombophilia may be congenital or acquired and is related to changes in haemostatic mechanisms with increased risk of thromboembolism also. In general, thrombophilia should be considered a multifactorial disorder and not as an expression of a single genetic abnormality.[7] The relationship between thrombophilic factors and infertility should be taken into consideration because of the possibility of alterations in haemostasis of a thrombophilic nature at the implantation site. This vascular change affects trophoblast invasion and placental vasculature, hampering implantation of the embryo. The cause of unexplained infertility needs to be assessed more thoroughly so that we can treat more and more cases with specific treatment. If we can find out the cause more specific treatment can be provided and pregnancy rate can be improved. So far there have been very few studies conducted to find out the association of unexplained infertility with congenital thrombophilia so we conducted this study. Congenital factors suspected of being responsible for propensity for thrombosis include protein C, Protein S, Activated protein C resistance (APCR). Also, a great proportion of theses are due to factor V Leiden mutation. Increased homocystein levels may be acquired or genetic. Genetic may manifest due to Methylene tetrahydrofolatereductase (MTHFR) gene mutation, Antithrombin 111 and Prothrombin 20210 gene mutation. The aims of this study was to screen for congenital thrombophilia in unexplained infertility, and compare the incidence of thrombophilia with fertile female population to find whether it is one of the possible causes of unexplained infertility and if it is so, to determine the type of thrombophilia more prevalent in unexplained infertility.

METHODOLOGY

Study was conducted at All India Institute of Medical Sciences, New Delhi, India. After ethical clearance which was obtained from the Ethics Committee of the institute. Patients were recruited from the out-patient clinic after confirming the diagnosis of unexplained infertility. Informed written consent was taken after explaining the detailed plan and purpose of the study in their own language. Study group (Group A) comprised of 40 infertile patients of age between 20-40 years in whom no cause of infertility was identified. The women with male factor infertility, tubal, ovarian, uterine factors like PCOD, tubal block, tubercular endometritis contributing to infertility and history of thrombosis were excluded. The control group comprised of 40 healthy women with proven fertility, matched for age, with no history of thrombo-embolic events, who had conceived spontaneously and had at least one uneventful pregnancy, without any complication (such as preeclampsia, intrauterine growth restriction and intrauterine fetal death).

On enrollment a detailed clinical history including menstrual history, obstetric history and medical history was taken. Detailed examination including general physical examination and gynaecological examination was done. Baseline blood investigations like hemoglobin, VDRL, HIV, HBsAg, blood sugar-fasting/post prandial, thyroid stimulating hormone, prolactin, day-2 follicle stimulatinghormone (FSH), leutinising hormone (LH), estradiol, AMH (Anti-Mullerian

Hormone), husband semen Analysis (HSA) and a day-2 antral follicle count (AFC) were done. An ultrasound pelvis for accessing endometrium and ovaries, hysterosalpingogram for tubal status, and diagnostic laparoscopy-hysteroscopy were performed in all patients, before labeling them as unexplained infertility.

After proper selection of participants and informed consent, blood samples were collected for testing of congenital thrombophilic factors. Tests for inherited thrombophilia included Protein C, Protein S, Anti-thrombin III (Functional assay), Activated Protein C Resistance, serum Homocysteine, factor V Leiden (PCR), Prothrombin G20210 G (PCR) gene and MTHFR gene mutation, were done in both the groups. Protein C and Protein S are sandwich ELISA based tests, and Protein C and Protein S relative percent concentrations were determined against a curve prepared from the reference plasma provided with the kit. Anti-thrombin III is a chromogenic test. Activated Protein C Resistance is a plasma-based functional clotting assay. Factor V Leiden analysis was done if APCR was found to be low. Prothrombin 20210 was tested if Antithrombin was found to be lower to check point. And MTHFR gene was analysed in cases with higher homocysteine levels.

The primary outcome variables included incidence of congenital thrombophilia in unexplained thrombophilia and its comparison with control fertile female population and the frequency of congenital thrombophilia in the unexplained infertile population.

Mutation study of FVL, MTHFR and PT gene by PCR-RFLP method:

Genomic DNA was extracted from peripheral blood leukocytes by standard phenol chloroform methods. DNA amplification for FV Leiden, prothombin 20210G/A and MTHFR 677C/T was performed separately as individual PCR. Polymerase chain reaction was performed on PCR mixture containing total volume of 25µl that contained 200 ng DNA, 0.2 mmol/L of each of the deoxynucleotide triphosphate; 25 pmol of each oligonucleotide primer, each forward and reverse, reaction buffer (giving final concentration of 10 mmol/L, Tris-HCL, 50 mmol/L KCL, 1.5mmol/L $MgCl_2$, and 0.05% gelatin); and 1 U Taq polymerase. The PCR conditions were common for all the above PCRs. The conditions were 95^0 C for 5 minutes followed by 30 cycles at 95^0 C for 1 minute, 55^0C for 1 minute, and 72^0C for 10 minutes.

Statistical Analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used. Quantitative variables were compared using Unpaired t-test/Mann-Whitney Test and qualitative variables were compared using Chi-Square test /Fisher's exact test. Odds ratio with 95% confidence intervals calculated for selected variables and their significance tested. A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

This is a cross-sectional study conducted to evaluate the incidence of congenital thrombophilia in unexplained infertility and to compare its incidence with the normal fertile population. A total of 40 participants were taken in the unexplained infertility group and 40 controls were taken. Participants in the control group were healthy females who had being borne at least one child.

The demographic profile was comparable in the two groups. The mean age of participants, BMI, mean FSH The mean prolactin, mean TSH level, mean E2 levels, mean AMH level were comparable in the unexplained infertility and control group (table 1)

Table 1: Comparison of demographic features in Unexplained Infertility Group and Control group

Demographic details	Group 1 (Unexplained Infertility)	Group 2 (Control)	P-value
Age[a]	29.4 +/- 3.04	28.52+/-3.18	0.09
Height[a]	160.25+/-3.36	160.28+/-3.25	0.992
BMI[a]	22.6+/-1.41	23.47	0.132
Hemoglobin[a]	11.59+/-0.97	11.06+/-1.02	0.12
Fasting blood sugar[a]	91.3+/-11.89	109.72+/-15.75	0.06
TSH[a]	2.57+/-1.02	2.45+/-0.91	0.985
FSH[a]	5.8+/-1.4	5.61+/-1.13	0.504
LH[a]	4.95+/-1.44	5.04+/-2.25	0.661
PRL[a]	13.99+/-2.75	15.26+/-2.2	*0.01*
E2[a]	90.64+/-17.91	92.13+/-12.17	0.743
AMH[a]	3.54+/-1.26	4.1+/-0.9	*0.026*
Day 2 AFC	9	8	0.186

a = mean ± Standard Deviation, b = median (maximum-minimum)

There were four (10%) patients in the unexplained infertility group (A) and one (2.5%) patient in the control group with low protein S. (p value-0.199). There were three (7.5%) patients with low Protein C in the infertility group and one (2.5%) in the control group (p value-0.328).

It was found that there were four (10%) subjects with low antithrombin levels in the infertility group and none in the control group. (p value- 0.127). Prothrombin gene mutation was studied in the four subjects who had low levels of Antithrombin III and it was found that two of them tested positive for the Prothrombin gene in study group.

It was found in this study that there were two (5%) cases with low APCR levels in the infertile population and two (5%) women with low APCR levels in the control population. (p value-1.000). Factor V Leiden mutation analysis was done for the four cases with low APCR levels and it was found that all four (two in study and two in control) tested positive for mutation (p value-1.000). All four were heterozygous for the mutation. Three (7.5%) cases from study group were reported to have high homocysteine levels and in the control population, one (2.5%) had high homocysteine level (p value-0.651). MTHFR mutation was tested in four cases with high homocysteinelevels ,3(all from the study group) were found positive for the mutation. Incidence of various inherited thrombophilia component in both groups are depicted in table 2

Table 2: Frequency of deficiency of thrombophilia factors in Unexplained Infertility group and Control group

	Unexplained Infertility N=40, no (%)	**Control Group N=40, no (%)**	**P-value**	**Odds ratio**	**95% CI**
Protein S	4 (10)	1 (2.5)	0.199	4.333	0.4624-40.6101
Protein C	3 (7.5)	1 (2.5)	0.328	3.162	0.3147-31.7768
Antithrombin III	4 (10)	0 (0)	0.127	9.986	0.5196-191.9154
Homocysteine	3 (7.5)	1/40 (2.5) 39/40 (97.5)	0.328	3.162	0.3147-31.7768
Activated Protein C resistance	2 (5)	2 (5)	1.000	1.000	0.1339-7.4702
Factor V Leiden Mutation	2 (5)	2/(5)	1.000	1.000	0.1339-7.4702
Prothrombin Gene Mutation (in Antithrombin III deficiency)	2 (2.5)	0 (0)	0.289	5.260	0.2446-113.1126
MTHFR Mutation (In cases with high homocysteine)	3 (7.5)	0 (0)	0.186	7.560	0.3778-151.293

Though, individually thrombophilic factors were not found to be significantly different in two groups, when a combined analysis was done, it was found that study group had significantly more thrombophilia cases. In the study group, there were 23 thrombophilia screen positives components whereas only seven abnormal thrombophilia factors were detected in 5 cases in control (p value-0.005). It was found that out of 23 positive thrombophilia factors in 14 cases in group 1, Protein S (10%) and Antithrombin III (10%) had the highest frequency followed by Homocysteine (7.5%), Protein C (7.5%), MTHFR gene mutation (7.5%), prothrombin 20210 (5%), Factor V Leiden (5%) and APCR (5%). Out of the seven positive thrombophilia cases in the control population, Factor V Leiden mutation and APCR had the highest frequency (5%) followed by Protein S, Protein C, and Homocysteine (2.5%); whereas, there were no cases of Anti-thrombin III and so Prothrombin 20210, and MTHFR gene. Overall there were 14 (35%) subjects in the unexplained infertility group with thrombophilic factor positive and 5 (12.5%) in the control group with positive thrombophilic factor (p value-0.023).

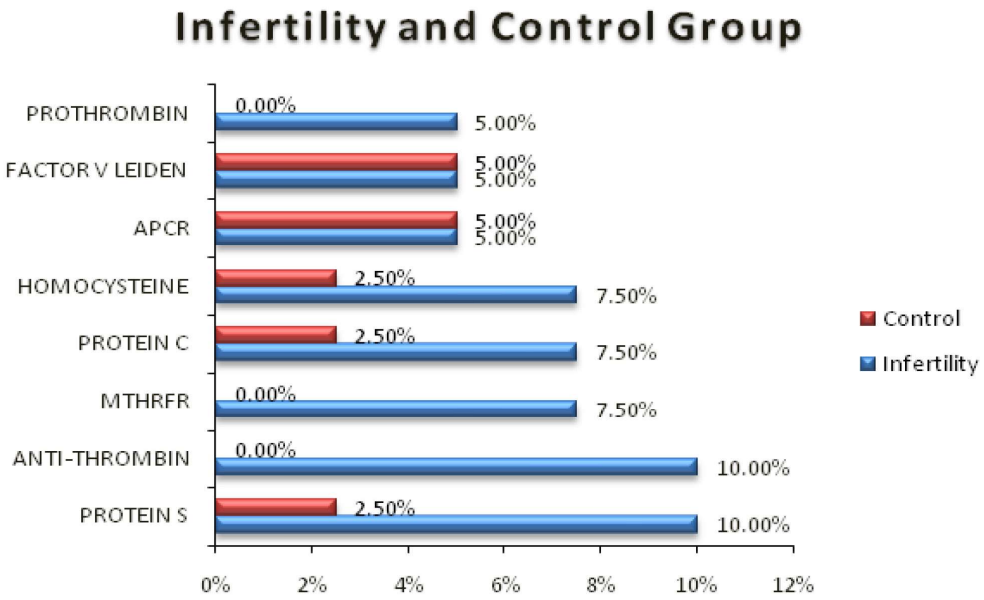

Fig 1: Graph showing the frequency of different Thrombophilias in the Unexplained Infertility group

In the study group, 5 cases had single factor deficiency/derangement; whereas, 7 cases had two and 2 cases had three factors deranged/ deficinet. In the control group, there were two patients with combined thrombophilias (two thrombophilic abnormalities). One patient had Protein C deficiency with hyperhomocysteinemia and the other patient had Protein S deficiency with hyperhomocysteinemia. (Details of thrombophilia screen factors is outlined in table 3)

Table 3: No of total cases and various factors in both group

Thrombophlia	Unexplained Infertility group Cases 40, tests 209	Control group Cases 40, tests 203	P-value
Thrombophilia positive cases	14 (35%)	5 (12.5%)	0.023
Total thrombophilia factors	23 (12%)	7 (3.4%)	0.005
Combined factors	1 in 7, 2 in 5, 3 in 2	1 in 3, 2 in 2	-

Table 4: Combined thrombophilia prevelence

Total no of Thrombophilia Factors	Positive cases-unexplained infertility, n=14	Positive cases-Control group n=5
One factor	7 (50% of 35%)	3 (60% of 12%)
Two factors	5 (a, b, c)	2 (c)
Three factors	2 (d,e)	0

a. Hyperhomocysteinemia + MTHFR gene mutation (n=3)
b. Antithrombin 111 + Prothrombin gene mutation (n=2)
c. APCR deficiency + Factor V ladein mutation (n=2 in infertile and 2 in controls)
d. Low Protein S + Hyper- homocysteinaemia + MTHFR gene mutation (n+1)
e. Low Protein C + Hyper-homocysteinaemia + MTHFR gene mutation (n+1)

DISCUSSION

The association between thrombophilia and recurrent pregnancy loss or poor pregnancy outcome

is well known. It may act by impairing the initial vascularization process occurring at implantation, which is necessary for a successful pregnancy.[8-10] However, there are limited data on the association between thrombophilia, hereditary or acquired, with female infertility and IVF failure.[4,8,11] A possible connection between inherited thrombophilia and the etiology of infertility has been found in some studies.[12-15] Thrombosis in the placental vessels leads to hypo perfusion of the inter-villous space and may cause placentation failure.[16,17] Failures of implantation and early placentation of embryos in IVF may be caused by similar mechanisms. However, other mechanisms may be responsible too, like the damage of decidual or chorionic vessels, or reduction of trophoblast invasiveness.[18,19] This study aimed to find the prevalence of congenital thrombophilia in unexplained infertility and its comparison with normal fertile female population. We wanted to determine if inherited thrombophilia can be addressed as defect leading to unexplained infertility; hence, instead of expectant or empirical treatment, we can offer appropriate treatment to these cases of unexplained infertility to achieve more promising outcomes.

We found higher incidence of inherited thrombophilia in infertile females as compared to fertile ones (35% vs 12.5%); thus, nearly threefold higher incidence of inherited thrombophilias in unexplained infertility. (p=0.023) In our study, the prevalence of low Protein S in unexplained infertility group was 10% vs 2.5% in the control group. This was 4-fold higher than the normal population but was not statistically significant (p =0.199). Similar pattern was observed in Protein C deficiency; 7.5% and 2.5% in the unexplained infertility and control groups, respectively, (p-0.328). Comparable results are reported in previous trials. Azem et al found the incidence of low Protein S in the study group (8.9%), but none reported in the control group , but no participants in the either group had deficiency of Protein C.[8] Other studies have also found (Safdarian et al., Qublan) low Protein C (but statistically not significant) in the unexplained infertility a.

We found similar incidence of low APCR levels and Factor V Leiden mutation in both groups, as seen in various previous reports also (4,8,11,22), Hyperhomocysteinemia, and MTHFR gene mutation have also not been observed associated with infertility in some studies.[4,8,11,20,22,23] Whereas higher incidence of heterozygous Factor V Leiden, (p=0.001) and MTHFR C667T homozygous gene mutation (p=0.02) were found associated with unexplained infertility.[24] In another study, prothrombin gene G20210 was found associated with infertility as compared to fertile females (5.7% vs 2.1%)(p=0.04).[25] Some could not establishead higher prevalence of thrombophilia in unexplained infertility.[27]

In our study, there were 14 subjects in the Unexplained Infertility group and five in the control group with at least one positive thrombophilic factor (p value<0.023). There were a total of 23 positives factors in the unexplained Infertility group and seven in the control group (p value-0.005). In a study conducted by Safdarian et al. it was seen that the combine thrombophilia prevalence was 59 (61.5%) in the recurrent IVF failure group and 31 (32.5%) in the control group.[20] Qublan et al. conducted a study where they found that the prevalence of combined thrombophilia in the recurrent IVF failure group was 35.6%, which was similar to our study; and that in the control population was 4.4%, which was statistically significant.[21] So it can be stated that Inherited thrombophilia might play a role in early implantation failure of the embryo and may lead to undiagnosed pregnancy loss. These results may be extrapolated to unexplained infertility.

Most of the studies conducted till date have observed the incidence of congenital thrombophilia in Recurrent IVF failures cut very few studies have assessed prevalence of congenital thrombophilia in unexplained infertility. The results of the present study revealed a higher prevalence of inherited thrombophilia in the unexplained infertility women compared to normal fertile. Hence, it can be hypothesized that Inherited thrombophilia might play a role in the mechanism involved in early undiagnosed pregnancy loss which may be a cause for unexplained infertility also.

Overall, we observed that congenital thrombophilia is significantly more in infertile cases than normal females, and we could find strong association with Antithrombin III and prothrombin gene mutation. Hyperhomocysteinemia without MTHFR gene mutation is unlikely to lead to infertility, however if associated with MTHFR mutation (heterozygous or homozygous), it may be implicated for infertility. Factor V Leiden which were in 5% cases and heterozygous in both the groups, could not be considered as contributing factor for infertility.

Strength of present study is that it is one of the first study conducted in South Asia, to study the prevalence of Congenital Thrombophilia as a factor contributing to unexplained Infertility. The few studies, which have studied congenital thrombophilia, have not studied all the congenital

thrombophilia factors. They have only studied the DNA mutation factors, which are inherited and are a cause of thrombophilia. The limitation of the present study is the small sample size. A larger sample size would have helped in determining prevalence of the thrombophilias in a more precise manner. Another drawback is that no intervention was given for the factors, which had come out to be positive in the unexplained infertility group. An intervention would have helped to find out, whether a prophylactic treatment may aid in conception in women with unexplained infertility.

CONCLUSION

Inherited thrombophilia combined analysis showed increased association of unexplained infertility with thrombophilia. Evaluation of thrombophilias may be an additional evaluation, beside other basic evaluations in patients with unexplained infertility. Inherited thrombophilia may have a significant role in embryo implantation failure. wProspective randomized controlled interventional studies with large numbers are needed to prove this effect and determine the effect of thromboprophylaxis in such cases.

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