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Methylene Tetra-Hydrofolate Reductase Gene Polymorphism and Endometrial Perfusion in Unexplained Female Infertility

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Abstract

Aim of the study: Examing the role of Methylene tetra-hydrofolate reductase (MTHFR C677T) polymorphisms in unexplained female infertility. *Methods*: The study was conducted on women with unexplained infertility attending the Infertility Clinic at El-Shatby University Hospital, Alexandria, during the period from October 2020 to October 2021. Uterine artery Doppler assessment and detection of MTHFR C677T gene mutation were done. The frequencies of homozygous and heterozygous gene mutations were determined. *Results*: In group I, 35 cases had abnormal uterine artery Doppler compared to 22 normal cases in group II. As regards MTHFR C677T gene mutation, 19 cases were positive in group I (7 were homozygous and 12 were heterozygous) and only one case was positive in group II (heterozygous) which was statistically significant. *Conclusion*: MTHFR C677T gene polymorphisms may play a role in unexplained infertility.

Keywords

Unexplained Infertility, Uterine Doppler, MTHFR Gene Polymorphism

1. Introduction

Unexplained infertility is defined as failure of a couple to achieve pregnancy without presence of definite cause after 12 months of unprotected intercourse, or after 6 months in female more than or equal to 35 years old [1]. No definite cause for infertility could be detected in ten to twenty five percent (10% - 25%) of infertile females [2].

Embryo implantation and good pregnancy rates require good uterine blood

flow. Low pregnancy rate, poor outcome and unexplained infertility were attributed to higher uterine arterial resistance which can be assessed by Doppler ultrasound [3] [4] [5] [6].

Thrombophilic factors are considered to hamper fertility and to interfere with implantation suggesting a negative effect of hypercoagulability on embryo implantation [7].

Nevertheless, the relationship between thrombophilia, including the MTHFR C677T mutation, and infertility has not been proved yet.

According to these data, we conducted the present study to investigate the role of MTHFR C677T mutation in infertile women having abnormal uterine artery blood flow.

2. Materials and Methods

The study was conducted on women with un-explained infertility attending the infertility Clinic at El-Shatby University Hospital, Alexandria, during the period From October 2020 to October 2021 and satisfying the following criteria:

Inclusion criteria

- Age: less than 30 years.
- Regular cycle and normal levels of FSH, LH, TSH, prolactin, serum androgens and AMH.
- Normal hysterosalpingogram.
- Normal semen analysis.
- No history of systemic or gynecological diseases that cause infertility.
- Women did not use intrauterine devices or oral contraception.

Exclusion criteria

- Age: more than 35 years old.
- History of systemic or gynecological diseases.
- Use of intrauterine device or hormonal contraception.
- Patient refusing the consent.

All participants signed an informed consent and the study was approved by the ethics committee of Alexandria faculty of medicine, Egypt.

They were subjected to thorough history taking, general examination, gynecological examination and investigations including; uterine artery Doppler assessment and detection of MTHFR C677T gene mutation.

Bilateral uterine artery Doppler was transvaginally assessed using Xario 200 ultrasound machine (Canon Medical System, Toshiba, Japan) with 7.5 MHz endovagianl probe during the midluteal phase. Initially, endometrial thickness was measured while the uterus was in the sagittal plane then, the transducer was moved laterally to evaluate the uterine artery at the point of crossing the external iliac vessels.

Doppler wave was activated, the angle of insonation was adjusted and three consequent similar waves of uterine artery flow were obtained. We assessed Doppler waveforms and measured resistance index (RI), the pulsatility index (PI), and the systolic/diastolic ratio (S/D) for both uterine arteries and the mean

values were calculated. Simultaneously we collected blood samples for detection of MTHFR C677T gene mutation. Under a complete aseptic technique, blood was withdrawn through venipuncture from antecubital vein in a vacutainer tube where 2 ml were emptied into a lavender tube [containing Ethylene-Diamine-Tetra-Acetic acid (EDTA)] MTHFR C677T gene mutation was assayed based on polymerase chain reaction (PCR) and reverse-hybridization [8]. The frequencies of homozygous and heterozygous gene mutations were determined.

3. Results

After a period of one year, we had 52 cases satisfying the selection criteria, 35 cases had increased uterine blood flow impedance diagnosed by high RI, PI, and S/D ratios (group I) and 22 cases had normal uterine artery blood flow (group II). The mean ages of both groups were 27.4 and 27.5 years respectively which was statistically nonsignificant (0.40 NS) (Table 1). The mean BMI was the same in both groups (26.7) which was also statistically nonsignificant (p = 0.49) (Table 1).

Both groups were tested for presence of the MTHR C677T gene mutation, in group I we had 19 positive cases compared to only one case in group II. Significant heterogeneity was found; in group I, we had 12 heterozygous cases compared to 7 homozygous ones and in group II the only positive case was heterozygous.

These results showed significant association between MTHFR C677T and abnormal uterine artery Doppler (X2 = 6.78, p = 0.009) (Table 2, Figure 1).

4. Discussion

Successful implantation of embryo in the uterus is mainly determined by ovum quality and receptivity of uterine endometrium [9]. Receptivity of the uterine endometrium is regulated by uterine artery blood flow as well as endometrial perfusion [10]. Increased impedance of uterine arteries' blood flow leads to poor growth of endometrium and endometrial thinning [11].

Table 1. Relation between demographic data and Doppler findings.

	Group I Abnormal Doppler "n = 35"	Group II Normal Doppler "n = 22"	P value
Age			
Range	24 - 30	24 - 30	
Mean	27.4	27.5	0.40 N.S.
SD	1.8	1.9	
BMI			
Range	20 - 30	22 - 32	
Mean	26.7	26.7	0.49 N.S.
SD	3.3	2.7	

N.S.: statistically not significant (a p-value more than 0.05).

Table 2. Relation between mother gene mutation and Doppler findings.

Positive MTHFR gene mutation (n = 19)	Group I Abnormal Doppler "n = 35"		Group II Normal Doppler "n = 22"		X^2 P value
	No	%	No	%	
Heterozygous					
Positive	12	34.3	1	4.5	6.78
Negative	23	65.7	21	95.5	0.009*
Homozygous					
Positive	7	20.0	0	0.0	FET
Negative	28	80.0	22	100.0	0.036*

FET = Fisher exact test. *: statistically significant (a p-value less than 0.05).

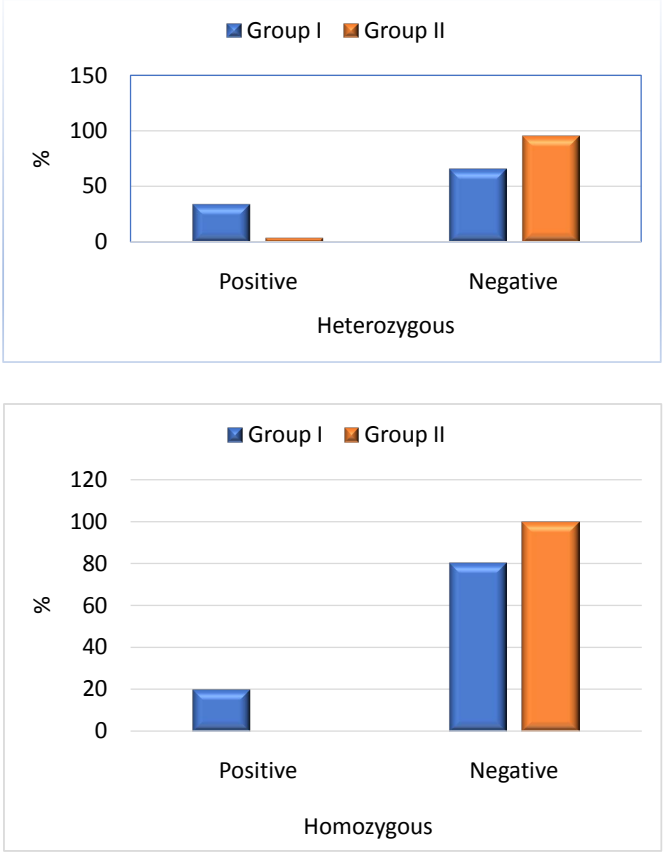

Figure 1. Relation between mother gene mutation and Doppler findings. % of cases having the mutation is plotted on the vertical axis and the genotype (homozygous or heterozygous) is plotted on the horizontal axis.

In recent years, a relationship between the thrombophilia factor and low fertility in women has been suggested. Several genes involved in the regulation of the endometrial receptivity were discovered [12] [13]. Mutations (homozygous and heterozygous) in the MTHFR (C677T and A 1298C) gene decrease the MTHFR enzyme activity and stability impairing folic acid metabolism and

causing hyper-homocysteinaemia which results in increased coagulation tendency. This mechanism could lead to thrombosis of maternal vessels impairing the perfusion of the endometrium and impairing implantation [14] [15].

Accordingly, researchers were eager to investigate the presence of the MTHFR C677T mutation in infertile women and in women with failed implantation; however, the results are controversial. Some authors failed to identify inherited thrombophilia, including the MTHFR C677T mutation, as a risk factor for infertility, [16] [17] while others reported conflicting findings, particularly in women with implantation failure [18].

In a study done by Ge J and others, recurrent early miscarriage was higher among patients with either inherited or acquired thrombophilia defects [19]. These results were explained by possible reduction in the perfusion of the intervillous space leading to placentation failure which could be due to thrombosis of maternal vessels.

In a meta-analysis including different ethnic subgroups, the association between MTHFR polymorphisms and unexplained early pregnancy loss (URPL) was examined. Researchers found a significant association between MTHFR C677T mutation and URPL only in the East Asian subgroup [20].

Results from other studies were completely different, some researchers demonstrated that the alleles of MTHFR C677T and A1298C were not associated with implantation failure and recurrent miscarriage. The same results were reported by Holmes *et al.*

Another study included 19 singleton pregnancies with abnormal blood flow in the uterine arteries during the first trimester of gestation compared to 24 matched control with normal flow patterns. All patients were genotyped for sequence variations in different thrombophilic genes including MTHFR. No differences were found between groups in any of these genes. Researchers also reported that the co-occurrence of several polymorphisms in the same patient was also not related to the blood flow patterns in the uterine arteries.

All these studies investigated MTHFR C677T mutation in cases of either failed implantation or recurrent early pregnancy loss due to poor uterine perfusion but studies including infertile cases concentrated only on presence or absence of normal uterine blood flow assessed by Doppler ultrasonography without investigating the possible cause of abnormal uterine artery Doppler. Considering unexplained infertility a result of failed implantation in a couple having no definite cause of failed conception, we could compare our results to studies investigating failed implantation and early pregnancy loss. Accordingly, their results could partially match with our results as we found only one case with positive mutation in the group with normal uterine artery Doppler which was statistically insignificant, but we found a significant difference in the group with abnormal uterine artery Doppler.

5. Conclusion

MTHFR C677T gene mutation may play a vital role in unexplained infertility.

Recommendation

Investigating thrombophilia may be an additional evaluation beside other host evaluations in patients with unexplained infertility.

Limitations of the Study

The result of our study is limited because of the case-control nature of the study and large prospective investigations are warranted to confirm this association before embarking in screening or intervention studies.

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Ethical Approval

By the *Ethical Committee of Alexandria Faculty of Medicine* (*Approval Number*: 2018-117990).

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] El-Shourbagy, S., Ossman, A.M.E. and El-Mohamady, A. (2017) The Role of Sildenafil Citrate (Viagra) Suppositories on Endometrial Response (Thickness and Mean Resistance Index of Endometrial Spiral Artery) in Cases of Unexplained Infertility. *Menoufia Medical Journal*, **30**, 343-349. https://doi.org/10.4103/1110-2098.215451

[2] Fenning, N.J., Campbell, B.K., Kendall, N.R., Clewes, J.S. and Johnson, I.R. (2004) Endometrial and Subendometrial Perfusion Are Impaired in Women with Unexplained Subfertility. *Human Reproduction*, **19**, 2605-2614. https://doi.org/10.1093/humrep/deh459

[3] Lilic, V., Pavlovic-Tubis, A., Radovic-Janosevic, D., Petric, A., Stefanovic, M. and Zivadinovic, R. (2007) Assessment of Endometrial Receptivity by Color Doppler and Ultrasound Imaging. *Medicinski Pregled*, **60**, 237-240. https://doi.org/10.2298/MPNS0706237L

[4] Fanchin, R. (2001) Assessing Uterine Receptivity in 2001: Ultrasonographic Glances at the New Millennium. *Annals of the New York Academy of Sciences*, **943**, 185-202. https://doi.org/10.1111/j.1749-6632.2001.tb03802.x

[5] Steer, C.V., Campbell, S., Pampiglione, J.S., Kingsland, C.R., Mason, B.A. and Collins, W.P. (1990) Transvaginal Colour Flow Imaging of the Uterine Arteries during the Ovarian and Menstrual Cycles. *Human Reproduction*, **5**, 391-395. https://doi.org/10.1093/oxfordjournals.humrep.a137109

[6] Goswamy, R.K., Williams, G. and Steptoe, P.C. (1988) Decreased Uterine Perfusion—A Cause of Infertility. *Human Reproduction*, **3**, 955-959. https://doi.org/10.1093/oxfordjournals.humrep.a136825

[7] Simon, A. and Laufer, N. (2012) Assessment and Treatment of Repeated Implantation Failure (RIF). *Journal of Assisted Reproduction and Genetics*, **29**, 1227-1239. https://doi.org/10.1007/s10815-012-9861-4

[8] Bowen, D.J., Bowley, S., John, M. and Collins, P.W. (1998) Factor V Leiden (G1691A), the Prothrombin 3'-Untranslated Region Variant (G20210A) and Thermolabile Methylenetetrahydrofolate Reductase (C677T): A Single Genetic Test Genotypes All Three Loci—Determination of Frequencies in the S. Wales Population of the UK. *Thrombosis and Haemostasis*, **79**, 949-954. https://doi.org/10.1055/s-0037-1615100

[9] Ng, E.H., Chan, C.C., Tang, O.S., Yeung, W.S. and Ho, P.C. (2007) The Role of Endometrial Blood Flow Measured by Three-Dimensional Power Doppler Ultrasound in the Prediction of Pregnancy during *in Vitro* Fertilization Treatment. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **135**, 8-16. https://doi.org/10.1016/j.ejogrb.2007.06.006

[10] Edi-Osagie, E.C., Seif, M.W., Aplin, J.D., Jones, C.J.P., Wilson, G. and Lieberman, B.A. (2004) Characterizing the Endometrium in Unexplained and Tubal Factor Infertility: A Multiparametric Investigation. *Fertility and Sterility*, **82**, 1379-1389. https://doi.org/10.1016/j.fertnstert.2004.04.046

[11] El-Toukhy, T., Coomarasamy, A., Khairy, M., Sunkara, K., Seed, P., Khalaf, Y., *et al.* (2008) The Relationship between Endometrial Thickness and Outcome of Medicated Frozen Embryo Replacement Cycles. *Fertility and Sterility*, **89**, 832-839. https://doi.org/10.1016/j.fertnstert.2007.04.031

[12] Celik, O., Unlu, C., Otlu, B., Celik, N. and Caliskan, E. (2015) Laparoscopic Endometrioma Resection Increases Peri-Implantation Endometrial HOXA-10 and HOXA-11 mRNA Expression. *Fertility and Sterility*, **104**, 356-365. https://doi.org/10.1016/j.fertnstert.2015.04.041

[13] Vannuccini, S., Clifton, V.L., Fraser, I.S., Taylor, H.S., Critchley, H., Giudice, L.C. and Petraglia, F. (2016) Infertility and Reproductive Disorders: Impact of Hormonal and Inflammatory Mechanisms on Pregnancy Outcome. *Human Reproduction Update*, **22**, 104-115. https://doi.org/10.1093/humupd/dmv044

[14] Chatzidimitriou, M., Chatzidimitriou, D., Mavridou, M., Anetakis, C., Chatzopoulou, F., Lialiaris, T. and Mitka, S. (2017) Thrombophilic Gene Polymorphisms and Recurrent Pregnancy Loss in Greek Women. *International Journal of Laboratory Hematology*, **39**, 590-595. https://doi.org/10.1111/ijlh.12703

[15] Enciso, M., Sarasa, J., Xanthopoulou, L., Bristow, S., Bowles, M., Fragouli, E., Delhanty, J. and Wells, D. (2016) Polymorphisms in the *MTHFR* Gene Influence Embryo Viability and the Incidence of Aneuploidy. *Human Genetics*, **135**, 555-568. https://doi.org/10.1007/s00439-016-1652-z

[16] Mousa, H.A. and Alfirevic, Z. (2000) Do Placental Lesions Reflect Thrombophilia State in Women with Adverse Pregnancy Outcome? *Human Reproduction*, **15**, 1830-1833. https://doi.org/10.1093/humrep/15.8.1830

[17] Rand, J.H., Wu, X.X., Guller, S., Scher, J., Andree, H.A. and Lockwood, C.J. (1997) Antiphospholipid Immunoglobulin G Antibodies Reduce Annexin-V Levels on Syncytiotrophoblast Apical Membranes and in Culture Media of Placental Villi. *American Journal of Obstetrics & Gynecology*, **177**, 918-923. https://doi.org/10.1016/S0002-9378(97)70294-1

[18] Vaquero, E., Lazzarin, N., Caserta, D., Valensise, H., Baldi, M., Moscarini, M., *et al.* (2006) Diagnostic Evaluation of Women Experiencing Repeated *in Vitro* Fertilization Failure. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **125**, 79-84. https://doi.org/10.1016/j.ejogrb.2005.08.001

[19] Ge, J., Wang, J., Zhang, F., Diao, B., Song, Z.F., Shan, L.L., Wang, W., Cao, H.J. and Li, X.Q. (2015) Correlation between MTHFR Gene Methylation and Pre-Eclampsia, and Its Clinical Significance. *Genetics and Molecular Research*, **14**, 8021-8028. https://doi.org/10.4238/2015.July.17.10

[20] Cao, Y., Xu, J., Zhang, Z., Huang, X., Zhang, A., Wang, J., Zheng, Q., Fu, L. and Du, J. (2013) Association Study between Methylenetetrahydrofolate Reductase Polymorphisms and Unexplained Recurrent Pregnancy Loss: A Meta-Analysis. *Gene*, **514**, 105-111. https://doi.org/10.1016/j.gene.2012.10.091