Research Article
Open Access

Lack of association between the occurrence of ectopic pregnancy and variations in the TGFβ1 and E-Catherin genes

Abstract

Purpose of the research: The aim of our study is to investigate the association between the impact of Transforming Growth Factor Beta-1 (TGFβ1) 29T/C and E-Cadherin-160C/A polymorphisms and occurrence of ectopic pregnancy.

Materials and methods: Our study was formulated as a case–control study where the genotype status of 95 women with a history of ectopic pregnancy were compared with 97 post-menopausal controls with at least two pregnancies and no history of ectopic pregnancy, with regards to their TGFβ1 29 T/C and E- Cadherin -160C/A polymorphism status. All blood samples were collected by standard peripheral venous puncture in EDTA containing tubes. DNA was extracted from peripheral blood leukocytes using standard procedures. Genotype status of the samples in their TGFβ1 and E-Cadherin genes was determined by Polymerase Chain Reaction (PCR) using specific primers designed to specifically amplify wild- type sequence, and detected by endonuclease digestion coupled to electrophoretic determination of the sequences. The differences in genotype and allele frequencies between the cases and normal controls were compared using statistical analysis. The differences in TGFβ1 and E-Cadherin genotype and allele frequencies between the cases and normal controls were compared using the Chi-Square test. Statistical significance with a P value lower than 0.05 was considered significant. Statistical analysis was performed using the SPSS software.

Principal results: Our analysis excluded the association of TGFβ1 29T/C and E-Cadherin 160C/A polymorphism with the occurrence of ectopic pregnancy. In this study, we did not observe and significant difference between the cases and controls in relation to the polymorphism of the TGFβ1 gene 29 T⁄C (P = 0.289) and E-Cadherin gene -160 C ⁄A (P = 0. 572). Similar conclusions were valid for the frequencies of other alleles as well.

Conclusion: Our study proved the lack of association between ectopic pregnancy and TGFβ1 29 T/C and E- Cadherin 160C/A polymorphisms. Based on our knowledge, this is the first report on the association of the TGFβ1 29 T/C and E-Cadherin 160C/A gene polymorphisms with ectopic pregnancy.

Keywords: Ectopic pregnancy, gene polymorphisms, TGFβ1, E-Cadherin

Volume 8 Issue 1 - 2019

Nurida Aghayeva,[1] Ali Benian,[1] İlhan Yaylım,[2] Saime Turan,[2] Akin Sevinc[3]

[1]Department of Obstetrics and Gynecology, Istanbul University, Turkey
[2]Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkey
[3]Department of Medicinal Biochemistry, Faculty of Medicine, Altinbas University, Turkey

Correspondence: Akin Sevinc, Department of Medicinal Biochemistry, Faculty of Medicine, Altinbas University, Istanbul, Turkey, Email akinsevinc@gmail.com

Received: November 28, 2018 | **Published:** January 08, 2019

Introduction

Ectopic pregnancy (EP) occurs when the developing blastocyst attaches to an undesired site in the uterine cavity, especially a location other than the endometrial region. Most commonly observed extra-uterine attachment location for the blastocyst is the fallopian tube, which unsurprisingly accounts for 98 percent of all ectopic gestations.[1] With the advance of our molecular understanding of EP, management of these pregnancies has changed dramatically over the recent years. Notwithstanding this advance in our understanding, it is important to note that hemorrhage caused due to ectopic pregnancy is still among the leading causes of pregnancy related maternal death in the first trimester and accounts for 4 to 10% of all pregnancy related deaths despite improved diagnostic methods leading to earlier detection and treatment.[1,2] Implantation of an embryo is a complex process that requires orchestrated interplay of numerous molecules and cellular pathways. The molecular interplay that takes place between the conceptus and the implantation site involves numerous intercellular and extracellular matrix interactions that are mediated by lectins, integrins, matrix degrading cumulus and their inhibitors, prostaglandins and various growth factors, cytokines and their receptors and other regulator proteins. Blastocyst implantation can occur only during a brief phase of receptivity that is known as the "implantation window", which encompass several systemic and local alterations termed "the maternal recognition of pregnancy". Albeit the extensive regulation of the molecular events in this phase, normal tubal epithelium may also possess a concurrent implantation window which may extend the opportunity for the trophoblast to attach a 5-to 7- day embryo in the tube.[3] Additionally, arsenal of molecular pathway profile of reproductive organs is somewhat unusual in regards to their proliferative and remodeling activities. For instance, local interplay of ovarian steroid estrogen and progesterone activity as well as several growth factors, cytokines and proteases manage the cyclic remodeling of human endometrium every 28 days. In accordance with its significant role in cell proliferation, differentiation, apoptosis and tissue remodeling activities, transforming growth factor (TGF) TGFβ1 superfamily member expression in the endometrium has been well documented.[4]

Considering the considerable amount of variation in the etiology of the ectopic pregnancy, we sought to interrogate the genetic variants that might contribute to the EP risk in Turkish population. For this purpose, we studied polymorphism of the TGFβ1 and E-Cadherin genes, since these genes code for major factors involved in maternal-fetal connection and communication during the establishment of pregnancy. TGFβ1 is a multifunctional cytokine and play a key role in reproduction.[4] Transforming growth factor superfamily members are abundantly and dynamically expressed in the endometrium, and appear, through their actions associated with cell proliferation, differentiation, apoptosis and tissue remodeling, to have instrumental roles in modulating cellular events involved in menstruation, proliferation, decidualization and the establishment of pregnancy.

TGFβ1 is a 25KDa homodimeric protein. The human TGFβ1 gene is located on chromosome 19q13, and contains seven exons. TGFβ1 is secreted in an active form[5] and as a latent complex in which TGFβ1 binds latency-associated peptide and latent TGF-β-binding protein. The production and secretion of TGFβ1 by epithelial glands in the secretory phase suggest roles in either the preparation of the endometrium for implantation, or direct actions on the pre-implantation embryo, facilitating development or differentiation for implantation. In accordance with this theory, TGFβ1 receptors are expressed by fallopian tube and uterine epithelial cells. Despite the importance of TGFβ1 in the regulation of pregnancy, the number of studies that analyzed the association of *TGFβ1* polymorphisms to RPL is far from expected levels.[6–8] The cell adhesion molecules are expressed on the surface of invasive trophoblasts, and these molecules interact with ligands expressed by the extracellular matrix of the decidua to control attachment and invasion.[9]

Accurate establishment of the intercellular adhesion linkages, cellular polarity, and tissue architecture is crucial for cellular functions. E-cadherin, a member of the Cadherin family of proteins, plays a central role in this function via its presence on both fetal and maternal membrane surfaces.[10] E-Cadherin has also been identified in human trophoblast populations where it mediates homophilic interactions between cytotrophoblasts.[11] In addition, E-Cadherin appears to play a role in modulating the expression and function of matrix metalloproteases (MMPs). Blastocyst invasion mimics several invasive characteristics with tumors, and MMPs are extensively involved in uterus tissue remodeling during embryo implantation. E-Cadherin regulates embryo implantation by decreasing the activity and expression of MMP-2 and MMP-9.[12]

E-cadherin gene (*CDH1*) which is localized on the long of the human 16th chromosome, has been shown to harbor some important polymorphisms, such as 160 C/A polymorphism on the promoter region which greatly influences the expression level of the *CDH1* gene.[13] Notwithstanding the presence of all these evidence, number of studies interrogating the role of *CDH1* gen polymorphisms in the success of implantation is limited,[14–16] where only a limited number of studies evaluate the expression of endometrial MMP-2 and -9 and E-Cadherin in peri- implantation phase of infertile women who have undergone in vitro fertilization cycles.[17] Despite the expression of MMP-2, MMP-9, and E-cadherin gene in the endometrium of infertile patients and the negative correlation of MMP-2, and a positive correlation of MMP-9 gene with E-cadherin gene expression, their results did not provide sufficient evidence for establishing a correlation between the expression of these molecules and the clinical IVF outcomes. In this study, we investigated the association of TGFβ1 and E-Cadherin SNPs with ectopic pregnancy in 192 Turkish women. Based on the literature review that we performed, this is the first comprehensive investigation on this subject.

Materials and methods

Prior to the study, Istanbul University Ethics Committee was obtained and all participants have given their given informed consent before participating in the study. Our study group consisted of 95 women with a mean age of 35.83 (range 21–65) years who were admitted to Department of Gynecology and Obstetrics, Istanbul University, Cerrahpaşa Faculty of Medicine ether with diagnosis of ectopic pregnancy or had a prior history of ectopic pregnancy. Our control group consisted of 97 women with a mean age of 57.96 years old (range 44–83), who is otherwise healthy, post-menopausal controls with at least two live births and did not any history of ectopic pregnancy. Two groups were matched by ethnic background of the patient. All blood samples obtained from the participants were collected in EDTA (ethylenediaminetetraacetate) containing tubes.

Genetic analysis

Peripheral blood samples from study and control groups were collected in 10 ml tubes containing EDTA and DNA isolation was carried out using salting-out technique. E-Cadherin 160 C/A (rs16260) and TGFβ-1 T29C (rs1800470) polymorphisms were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. The primers used for E-Cadherin 160 C/A gene region were forward 5'-GCC CCG ACT TGT CTC TCT AC-3' and reverse 5'-GGC CAC AGC CAA TCA GCA-3'. For the amplification reactions, PCR mixture of 25 µl volume was prepared for each sample for each gene containing approximately 100 ng of template, 0.5 µl of each primer, deoxyribonucleoside 5' triphosphate mixture (each at 0.2 mM), 2.5 mM MgCl2 and 1 U Taq polymerase in 1× reaction buffer (Intron Biotechnology). PCR conditions for these genes were: an initial melting step of 45 seconds at 95 ^{0}C ; followed by 35 cycles of 45 seconds at 94 ^{0}C , 45 seconds at 63 ^{0}C and 45 seconds at 72 ^{0}C ; and a final elongation step of 5 minutes at 72 ^{0}C .The restriction endonuclease *Tsp*45I was used to identify the presence or absence of -160 C/A polymorphism. The product of this reaction was characterized on agarose gels containing ethidium bromide. Presence of fragments on the gel image, that correspond to 364 and 83 bp after enzyme digestion would indicate that the sample was homozygous for the A allele (AA genotype). The homozygous individuals for C allele (CC genotype) were identified by the presence of 447 bp fragment, whereas the CA genotype of the gene was characterized by the presence of three fragments with sizes of 447, 364 and 83 bp.

The forward and reverse primers for TGFβ-1 T29C polymorphism were 5'-CCT CCC CACCAC ACC AG-3' and 5'- CCG CAG CTT GGA CAGG-3', respectively. PCR products were digested with *Msp*A1I restriction enzyme at 37 ^{0}C for 1 hour. After enzyme digestion, TT genotype was identified with the presence of 25, 40, 67 and 103 bp fragments after characterization of the restriction enzyme digestion products on gel electrophoresis. CC genotype was identified with the presence of 12, 25, 40, 67 and 91 bp products. The heterozygote TC genotype had 12, 25, 40, 67, 91 and 103 bp. Since it is not feasibly possible to determine 12 and 25bp fragments on agarose gel, and therefore genotypes were characterized using the other fragments

Results

Frequency distribution of genotypes and alleles for patient and control groups were calculated as percentages and are presented in Tables 1-3. Results clearly indicate the absence of a significant difference between the two groups. The TGFβ1 and E-Cadherin genotype distribution of each polymorphism in cases and controls were in Hardy–Weinberg equilibrium.

Table 1 Frequency of genotype polymorphisms for TGFβ1T29C in samples with ectopic pregnancy and control samples

			TGFβ1				P
			TT Genotype	TC Genotype	CC Genotype	Total	
Group	Ectopic	Number of Samples	21	54	20	95	0.289
		% within Group	22.10%	56.80%	21.10%	100.00%	
	Control	Number of Samples	30	45	22	97	
		% within Group	30.90%	46.40%	22.70%	100,0%	
Total		Number of Samples	51	99	42	192	
		% within Group	26.60%	51.60%	21.90%	100.00%	

Table 2 Frequency of genotype polymorphisms for e-Cadherin -160 c/a in ectopic pregnancy and control samples

			E-Cadherin				P
			CC Genotype	CA Genotype	AA Genotype	Total	
Group	Ectopic	Number of Samples	52	37	6	95	0.572
		% within Group	54.70%	38.90%	6.30%	100.00%	
	Control	Number of Samples	53	34	10	97	
		% within Group	54.60%	35.10%	10.30%	100,0%	
Total		Number of Samples	105	71	16	192	
		% within Group	54.70%	37.00%	8.30%	100.00%	

Table 3 Frequency of alleles for TGFβ1 T29C (rs1800470) and E-Cadherin -160 C/A (rs16260) in ectopic pregnancy and controls

TGFβ1	Ectopic: Control	P	Odds ratio (%95CI)	P	Odds ratio (%95 CI)	P	Odds ratio (%95CI)
T/T	21:30						
T/C	54:45:00	0.166	1.578(0.825-3.017)	0.785	1.1(0.555-2.182)	0.147	1.522(0.861-2.689)
C/C	20:22	[T/C+C/C versus T/T]		[T/T+T/C versus C/C]		[T/T+C/C versus T/C	
E-Cadherin							
A/A	6:10						
C/A	37:34:00	0.317	1.704(0.594-4.894)	0.989	0.996(0.564-1.758)	0.576	1.182(0.658-2.125)
C/C	52:53:00	[C/A+C/C versus A/A]		[A/A+C/A versus C/C]		[A/A+C/C versus C/A]	

Discussion

Notwithstanding the presence of significant amount of scientific data on its detection, management, and prevention numerous studies point to an increasing prevalence of ectopic pregnancy, which underlines that the significance of ectopic pregnancy has not diminished as a significant public problem. Ectopic pregnancy can lead to maternal deaths in the first trimester where most fatal cases result from delayed diagnosis and inappropriate investigation. Despite the identification of numerous risk factors for the development of ectopic pregnancy, their etiology and correspondence to the development of ectopic pregnancy has not been conclusive. For instance, several studies interrogated the relationship of TGFβ1 and E-Cadherin variants with the ectopic pregnancy pathogenesis, however, results of these studies has not been conclusive. An earlier study on a relatively limited number of subjects (n=8) documented TGFβ was localized immune histochemically in unruptured ectopic pregnancies removed by salpingectomy.[18] TGFβ1 receptors are expressed by fallopian tube and uterine epithelial cells.[19] The expression of TGFβ1 is influenced by several genetic determinants.[20,21] Several single-nucleotide polymorphisms throughout the TGFβ1 gene have been described; where some of them were associated with elevated TGFβ1 serum levels.[22,23]

Molecular biological evidence showed that polymorphisms in the TGFβ1gene that leads to Leu→ Pro substitution at amino acid 10, which includes a T→C transition at nucleotide 29 in the region encoding the signal sequence. However, the impact of the variant C allele in the TGFβ1 T29C polymorphism is still unclear. Hoffmann *et al*.,[24] showed reduced production of TGFβ1 protein with the variant C allele, while Dunning *et al*.,[25] demonstrated that the variant C allele was associated with higher TGFβ1 secretion *in vitro*. Based on the data we obtained from our study, we hypothesized that sequence variants that modify the expression profile of genes in the TGF-β signaling pathway may modify the ectopic pregnancy risk.

The E-Cadherin −160C/A SNP is located 160 bp upstream of the transcription start site of the E- Cadherin gene. It is well documented that the A allele decreases the transcriptional activity by 68% compared with the C allele in a reporter gene analysis, suggesting that the A allele may reduceE- Cadherin expression *in vivo*.[26] This finding is supported by other studies that reported similar reduced transcriptional activity from the A allele.[27] There are several studies on the association of −160C/A SNP with various cancer types (with gastric, prostate, bladder, breast, colorectal, nasopharyngeal, endometrial, pancreatic, cervical, lung, oral, liver, thyroid, and ovarian cancer and lymphoma), where results from these studies reveal that −160 SNP is a cancer type specific marker that also depends on the ethnic background of the patient.[28] While most studies on the −160C/A SNP focus on onset and progression of the cancer types mentioned above, a few have examined the association with numerous other diseases including orofacial clefts, asthma, urolithiasis, endometriosis and infection. Govatati *et al*.,[29] studied the association of −160 SNP with endometriosis in Indian women (715 cases who were diagnosed endometriosis and 500 control individuals) and found out that the −160A/A allele frequencies are statistically significantly higher in patients than in controls (<0.0019). Last but not least, a study performed among Japanese women (520 cases and 520 healthy controls) with this condition also pointed to the lack of association.[30] To the best of our knowledge, no study to date has interrogated the relationship between EP and TGFβ1 T29C and E-Cadherin -160 C/A gene polymorphisms simultaneously. Therefore, it is only possible to compare our results with other works that have studied the association of these polymorphisms with other diseases (cancers, postmenopausal osteoporosis, endometriosis and infection).

In our study, we did not observe significant variation between the cases and controls in relation to the polymorphism of the TGFβ1 gene T⁄C (P = 0.289), and E-Cadherin gene -160 C ⁄A (P = 0. 572). The same was observed for the allele frequencies. Therefore, it is logical to assume that genetic variability is not the sole factor affecting the risk for the presence of EP. Furthermore, it is important to note that our study was conducted in a Turkish patient population. It is well known that genotypes and allele frequencies may vary by population and ethnic background of the study population. Therefore, further studies with larger sample size that will include more diverse populations should be conducted in this field.

Acknowledgments

This study was supported by Research Foundation of Istanbul University, Project: 2462/50937.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Sivalingam VN, Duncan WC, Kirk E, et al. Diagnosis and management of ectopic pregnancy. *J Fam Plann Reprod Health Care*.2011;37(4):231–240.
2. Centers for Disease Control and Prevention (CDC). Ectopic pregnancy United States, 1990- 1992. *MMWR Morb Mortal Wkly Rep.* 1995; 44(3):46–48.
3. Sulz L, Valenzuela AM, Salvatierra ME, et al. The expression of alpha(v) and beta3 integrin subunits in the normal human fallopian tube epithelium suggests the occurrence of a tubal implantation window. *Hum Reprod.* 1998; 13(10):2916–2920.
4. Ingman WV, Robertson SA. The essential roles of TGFβ1 in reproduction. *CytokineGrowth Factor Rev.* 2009; 20(3):233–239.
5. Taylor AW. Review of the activation of TGF-b in immunity. *J Leukoc Biol*. 2009;85(1):29–33.
6. Prigoshin N, Tambutti M, Larriba J, et al. Cytokine gene polymorphisms in recurrent pregnancy loss of unknown cause. *Am J Reprod Immunol*. 2004;52(1):36–41.
7. Amani D, Dehaghani AS, Zolghadri J,et al. Lack of association between the TGF-beta1 gene polymorphisms and recurrent spontaneous abortion. *J Reprod Immunol.* 2005;68(1–2):91–103.
8. Von Linsingen R, Bompeixe EP, Bicalho Mda G. A case–control study in IL6 and TGFB1 gene polymorphisms and recurrent spontaneous abortion in southern Brazilian patients. *Am J Reprod Immunol*. 2005;53(2):94–99.
9. Lyall F. Mechanisms regulating cytotrophoblast invasion in normal pregnancy and pre- eclampsia. *Aust N Z J ObstetGynaecol.* 2006;46(4):266–273.
10. Rowlands TM, Symonds JM, Farookhi R, et al. Cadherins: crucial regulators of structure and function in reproductive tissues. *Rev Reprod.* 2000;5(1):53–61.
11. Coutifaris C, Kao LC, Sehdev HM, et al. E-Cadherin expression during the differentiation of human trophoblasts. *Development.* 1991;113(3):767–77.
12. Liu G, Zhang X, Lin H, et al. Effects of E-cadherin on mouse embryo implantation and expression of matrix metalloproteinase-2 and-9. *Biochem BiophysRes Commun.* 2006;343(3):832–838.
13. Nakamura A, Shimazaki T, Kaneko K, et al. Characterization of DNA polymorphisms in the E-Cadherin gene (CDH1) promoter region. *Mutation Research*. 2002;502(1–2):19–24.
14. Rahnama F , Thompson B, Steiner M, et al. Epigenetic regulation of E-Cadherin controls endometrial receptivity. *Endocrinology*. 2009;150(3):1466–1472.
15. Jha RK, Titus S, Saxena D, et al. Profiling of E-Cadherin, beta-Catenin and Ca2+ in embryo-uterine interactions at implantation. *FEBS Lett*. 2006;580(24):5653–5660.
16. Movaghar B, Askarian S. Expression of e-Cadherin, leukemia inhibitory factor and progesterone receptor in mouse blastocysts after ovarian stimulation. *Cell J*. 2012;14(3):225–230.
17. Maia-Filho VO, Rocha AM, Ferreira FP, et al. Matrix Metalloproteinases 2 and 9 and E-Cadherin Expression in the Endometrium During the Implantation Window of Infertile Women Before In Vitro Fertilization Treatment. *Reproductive Sciences*. 2015; 22(4): 416–22.
18. Selick CE, Horowitz GM, Gratch M, et al. Immunohistochemical localization of transforming growth factor-beta in human implantation sites. *J Clin Endocrinol Metab*. 1994;78(3):592–596.
19. Zhao Y, Chegini N, Flanders KC. Human fallopian tube expresses transforming growth factor (TGF- beta) isoforms, TGF -beta type I–III receptor messenger ribonucleic acid and protein, and contains TGF beta-binding sites. *J Clin Endoc Metab.* 1994;79(4):1177–1184.
20. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. *Hum Mol Genet*. 1999; 8(1):93–97.
21. Incebiyik A, Kocarslan S, Camuzoglu A, et al. Trophoblastic E-Cadherin and TGF-beta Expression in Placenta Percreta and Normal Pregnancies. *J Met-Fet & Neonat Med.* 2016;29(1):126–129.
22. Awad MR, El-Gamel A, Hasleton P, et al. Genotypic variation in the transforming growth factor-beta1 gene:association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation.* 1998;66(8):1014–1020.
23. Cambien F, Ricard S, Troesch A, et al. Polymorphisms of the Transforming Growth Factor-β1 Gene in Relation to Myocardial Infarction and Blood Pressure. *Hypertension.* 1996; 28(5):881–7.

24. Hoffmann SC, Stanley EM, Darrin Cox E, et al. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. *Transplantation.* 2001;72(8):1444-1450.

25. Dunning AM, Ellis PD, McBride S, et al. A transforming growth factor-beta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. *Cancer Res*. 2003;63(10):2610–2615.

26. Li LC, Chui RM, Sasaki M. A single nucleotide polymorphism in the E-Cadherin gene promoter alters transcriptional activities. *Cancer Res.* 2000; 60(4):873–876.

27. Cattaneo F, Venesio T, Molatore S. Functional analysis and case-control study of -160C/A polymorphism in the E-Cadherin gene promoter: association with cancer risk. *Anticancer Res*. 2006;26:4627–4632.

28. Wang L, Wang G, Lu C, et al. Contribution of the -160C/A polymorphism in the E-Cadherin promoter to cancer risk: a meta-analysis of 47 case-control studies. *PLoSONE*. 2012;7:7.

29. Govatati S, Tangudu NK, Deenadayal M, et al. Association of E-Cadherin single nucleotide polymorphisms with the increased risk of endometriosis in Indian women. *Mol Hum Reprod.* 2012;18(5):280–287.

30. K. Yoshida, K. Yoshihara, S. Adachi, et al. Possible involvement of the E-Cadherin gene in genetic susceptibility to endometriosis. *Human Reproduction*. 2012;27(6):1685–1689.