Experimental
&
Clinical Article

Obstetrics; *Maternal-Fetal Medicine and Perinatology*

Impaired Implantation and Hereditary Thrombophila; Expression of LIF (Leukemia Inhibitory Factor) on Extravillous Trophoblasts

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OBJECTIVE: LIF (Leukemia İnhibitory Factor) was shown to have an important role in implantation. The aim of this study is to investigate the expression of LIF on extravillous trophoblasts in normal pregnancies and pregnancies with MTHFR (methylenetetrahydrofolate reductase) mutations. This study was also designed to demonstrate the "impaired implantation based" perinatal complications such as early pregnancy losses in pregnancies with hereditary thrombophilia (MTHFR 677 & 1298 mutations; homocystinemia and impaired folat/vitamin B12 metabolisms).

STUDY DESIGN: Abortus material until 10th gestational-week were used in this study. The patients were divided into 2 groups as: Group 1; control group (unwanted induced abortions), Group 2; abortus material from pregnancies with hereditary thrombophilia. Hereditary thrombophilia cases were consisted of only MTHFR homozygote mutations (MTHFR 677 & 1298). Indirect ABC (avidin-biotin-peroxidase complex) was applied to all of the abortus material to investigate the expression of LIF.

RESULTS: Expression of LIF in extravillous trophoblasts was immünohistochemically stronger (+++) in MTHFR group than the extravillous trophoblasts of the control group (++). This finding was also found to be statistically significant ($p \leq 0.05$).

CONCLUSION: The impaired LIF expression of extravillous trophoblastic cells in MTHFR patients can be one of the reasons of early fetal losses due to impaired implantation. Direct affect of homocystinemia, cell degragates of maternal endothelial cells due to injury and thrombosis and activation of complement system may be the reasons of defective LIF synthesis. Defective LIF expression on extravillous (interstitial) trophoblasts may result in insufficient activation of the LIF receptors in the decidua and limiting their migration during placentation. On the other hand, number of the extravillous trophoblasts developing from cytotrophoblasts may also be negatively affected by the disturbed LIF expression resulting in a shallow placenta.

Key Words: MTHFR mutations, LIF, Miscarriages, Endometrial receptivity, Extravillous trophoblast

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Introduction

Implantation depends on the interaction between the blastocyst and the endometrium. During this interaction many molecules such as cytokins [Leukemia İnhibitory Factor (LIF), Colony-Stimulating Factor-1 (CSF-1), Interleukin-1 (IL-1)], growth factors and adhesion molecules (integrins & others) play important roles.[1,2]

LIF was first identified from its ability to induce differentiation of myeloid leukemia cells into macrophage-like cells. LIF is a highly glycosylated 40-50 kDa glycoprotein.[3] LIF secretion is controlled by steroid hormones, cytokines, and growth factors in the uterine environment.[4] It was shown that LIF is expressed in endometrial/glandular epithelium, stromal cells, decidual leukocytes, natural killer (NK) cells, different T- cell subtypes. LIF is also expressed in the preimplantation embryo.[2,5,6] LIF expression is important in preimplantation, implantation, embryo development and placentation.[7]

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme which takes an important role in folate metabolism and is encoded by the MTHFR genes.[8] Several MTHFR mutations such as C677T (the alanine- to- valine) and A1298C (the glutamate- to- alanine) were described.[9] It has been reported that fetal loss frequency is increased in pregnancies with MTHFR mutations[10]

Recently, there are many studies trying to explain the mechanisms of implantation in terms of molecular factors and cells that have roles in placentation.[1,2] The aim of this study is

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to investigate the role of LIF expression in implantation in patients with MTHFR mutations and try to compare them with normal pregnancies to find out the differences which may contribute to the defective implantation and fetal losses.

Material and Method

This study was approved by the ethical committee of Hacettepe University Faculty of Medicine, Ankara, Turkey (FON10/36). Consent forms were signed by all patients according to the regulations. In this study, abortion materials were collected from first trimester pregnancies until 10th gestational week. Materials were classified into two groups. Group 1; control group (Unwanted induced abortion material of healthy females) (n=8), Group 2; Spontaneous abortus materials from pregnancies with MTFHR mutations (n=8). After fixated in 10% buffered formaldehyde, endometrium samples were processed according to the routine light microscope tissue processing method and embedded in parafin.

LIF expression in the endometrium was evaluated by indirect ABC (avidin-biotin-peroxidase complex-Vectastatin) immunohistochemistry. 5 µm paraffin sections were deparaffinized in xylene, rapidly rehydrated through graded alcohol series, and immersed in 0.3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity. For the retrieval of antigens sections were heat-treated by boiling for 3 min in 10% EDTA buffer and washed in phosphate-buffered saline (PBS), pH 7.4. After incubation with 'ABC Kit Blocking Serum' for 20 minutes, LIF rabbit IgG antibody (1:200 dilution) was applied at room temperature one hour. In negative controls primary antibody is omitted and phosphate-buffered saline is applied for an hour. Sections were rinsed in PBS and incubated for 30 min with biotinylated secondary antibody. After being rinsed in PBS, the sections were incubated for 30 min with 'ABC Kit Reagent'. Sites of peroxidase activity in the sections were visualized with DAB as chromogen. The slides were counterstained with hematoxylin.

Degree of immunoreactivity was accepted as follow; (-): no immünoreactivity, (+): weak immunoreactivity, (++): moderate (mild) immünoreactivity, (+++): strong immünoreactivity. The degree of immunoreactivity was evaluated statistically by SPSS program (SPSS for Windows 15.0).

Results

Cytoplasmic LIF immunoreactivity was observed in the extravillous trophoblasts both in the control and MTHFR groups. Expression of LIF in extravillous trophoblasts was stronger in MTHFR group (+++) than the extravillous trophoblasts of the control group (++) (Figure 1,2,3). LIF immunoreactivity was statistically significant in the extravillous trophoblasts ($p \leq 0.05$).

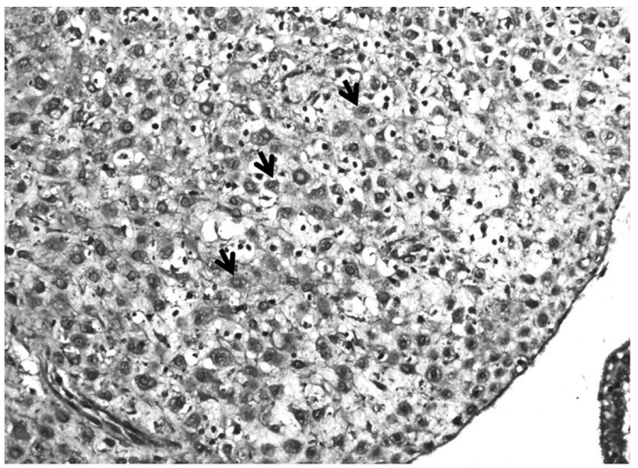

Figure 1: Control group: Mild cytoplasmic LIF expression in the extravillous trophoblasts (arrow) (ABC-peroxidase - Hematoxylin x200).

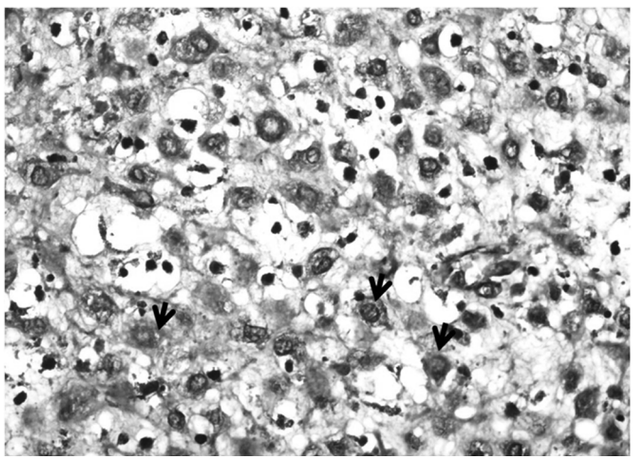

Figure 2: Control group: Mild cytoplasmic LIF expression in the extravillous trophoblasts (arrow) (ABC-peroxidase-Hematoxylin x400).

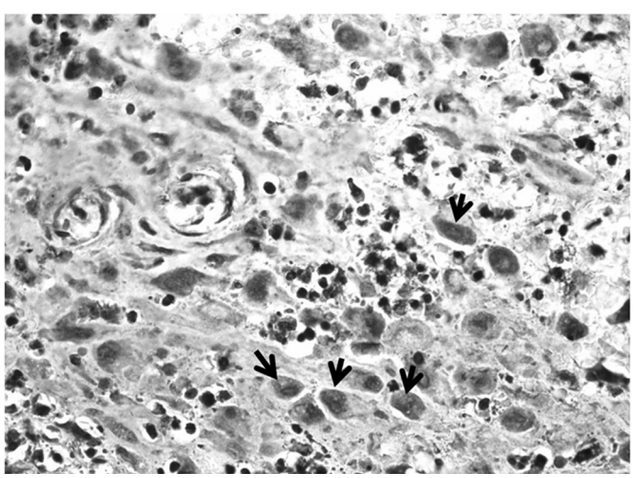

Figure 3: MTHFR group; Strong cytoplasmic LIF expression in the extravillous trophoblasts (Arrow) (ABC-peroxidase Hematoxylin x400)

Discussion

LIF has a regulatory role on trophoblasts. LIF is modulating the differentiation of cytotrophoblast to extravillous trophoblasts.[11]

In MTHFR mutations, receptors and extracellular matrix proteins in the decidual endometrium are affected and structural changes appear due to impaired homocysteine metabolism and forthcoming pathological events such as endothelial injury of placental vascular structures, cell degragates of endothelium, thrombus formation and activation of compleman system. In patients with MTHFR mutations, fetal anomalies are frequently seen because of the increased levels of tetrameric DNA due to disturbed folate metabolism.[12-16]

In this study, LIF expression observed in the cytoplasm of the extravillous trophoblasts was stronger (+++) in the MTHFR group than the control group (++), ($p \leq 0.05$). These findings have led us to speculate that, in patients with MTHFR mutations, LIF structure (or synthesis) in the extravillous trophoblasts may be impaired. This defective LIF may not be able to bind to its receptors (which may also be defective) inorder to enroll necessary biological functions. This may force the extravillous trophoblasts to produce more LIF resulting in a stronger cytoplasmic immunoreactivity in the extravillous trophoblastic cells. On the other hand, LIF which can not perform its function may accumulate in the cytoplasm increasing the cytoplasmic expression.

Patients with MTHFR mutations tend to experience perinatal complications such as recurrent fetal losses, intrauterine growth retardation (IUGR), preterm deliveries (especially going together with PPROM), preeclampsia, intrauterine hypoxia and ablatio placenta.[14-16] One of the manifestations of preeclampsia is shallow placenta (superficial placentation). It may be speculated that LIF may contribute to this disorder in two ways. Firstly, extravillous trophoblasts may not be able move deeper in the decidual endometrium because of the defective LIF, LIF receptors and/or defective relations with the extracelluler matrix proteins. Secondly, it was shown by other investigators that LIF is modulating the differentiation of cytotrophoblast to extravillous trophoblasts.[11] Impaired LIF synthesis may have a negative effect on the formation of extravillous (interstitial) trophoblasts from the cytotrophoblasts. Finally, the decreased number of extravillous trophoblasts may result in a shallow, defective placentation.

The results of this study implies that, the impaired LIF synthesis in extravillous trophoblasts and the improper activation of the LIF receptors in the decidua in pregnancies with impaired folate metabolism (MTHFR 677 &1298 mutations), can be one the reasons of defective placentation resulting in early pregnancy losses and other perinatal complications.

Plasentasyon Bozuklukları ve Herediter Trombofili; Ekstravillöz Trofoblastlarda Lif (Leukemia Inhibitory Factor) Ekpresyonu

AMAÇ: LIF (Leukemia İnhibitory Factor) implantasyonda önemli rol oynadığı bilinen bir moleküldür. Bu çalışmada, LİF'in normal ile MTHFR mutasyonu olan gebeliklerde ekstravillöz trofoblastlardaki ekspresyonu araştırıldı ve erken gebelik kayıpları ile herediter trombofili (MTHFR 677 & 1298 mutasyonları; homosistinemi ve folat/vitamin B12 metabolizma bozuklukları) arasındaki ilişkinin gösterilmesi planlandı.

GEREÇ VE YÖNTEM: Bu çalışmada ilk 10 gebelik haftasındaki abortus materyalleri kullanıldı. Hastalar, iki gruba ayrıldı: Grup 1; kontrol grubu (isteğe bağlı gebelik sonlandırılmaları), Grup 2; herediter trombofili hastalarına ait düşük materyali. Herediter trombofili hastaları grubuna yalnızca MTHFR homozigot mutasyonu (MTHFR 677 & 1298) olan gebeler dahil edildi. LIF expresyonunu incelemek için tüm gruplara ait olan materyale indirekt ABC (avidin-biotin-peroksidaz kompleks) uygulandı.

BULGULAR: Ekstravillöz trofoblastlarda sitoplazmik olarak gözlenen LIF ekspresyonu, MTHFR grubunda (+++) kontrol grubuna göre (++) daha kuvvetliydi. Bu bulgu istatistiksel olarak da desteklendi ($p \leq 0.05$).

SONUÇ: MTHFR hastalarında görülen erken fetal kayıpların nedenlerinden biri de ekstravillöz trofoblastlarda LIF ekspresyonunun bozulmasına bağlı olarak ortaya çıkan implantasyon yetersizliği olabilir. Homosistinemi, tromboz, kompleman sisteminin aktive olması gibi nedenlerle zedelenen maternal endotel hücrelerinden açığa çıkan yıkım ürünleri ekstravillöz trofoblastlarda LIF sentezini bozabilir. Ekstravillöz (interstisyel) trofoblastlarda LIF ekspresyonunun bozulması da desidua da LIF reseptörlerinin yetersiz aktivasyonuna yol açabilir. Sonuçta, bu hücrelerin göç yeteneklerinin olumsuz etkilenmesi de yetersiz plasentasyona neden olabilir. Ayrıca, LIF ekspresyonunun bozulması, sitotrofoblastlardan ekstravillöz trofoblastların farklanmasını da olumsuz etkileyebilir. Plasentasyon yetersizliğine bu hücrelerin sayıca yetersizliği de katkıda bulunabilir.

Anahtar Kelimeler: MTHFR mutasyonları, LIF, Erken gebelik kaybı, Endometriyum reseptivitesi, Ekstravillöz trofoblast

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