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Impaired Placentation and Early Pregnancy Loss in Patients with MTHFR Polymorphisms and Type-1 Diabetes Mellitus

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ABSTRACT

Objective: To evaluate the impact of type-1 diabetes mellitus (DM) and methylenetetrahydrofolate reductase (MTHFR) polymorphisms on impaired placentation leading to early pregnancy loss. **Methods:** Miscarriage materials were obtained from eight pregnant women with type-1 DM without MTHFR polymorphism, eight with MTHFR polymorphisms without type-1 DM, and eight controls with neither DM nor MTHFR polymorphisms. Insulin-like growth factor-1 (IGF-1), leukemia inhibitory factor (LIF), and Beclin-1 expression were assessed to evaluate placentation. **Results:** Cytoplasmic LIF, IGF-1, and Beclin-1 expression were decreased in the superficial and glandular epithelial cells of the decidua in both study groups. LIF expression was increased in interstitial trophoblasts in the MTHFR group. IGF-1 expression was decreased in the decidual cells and interstitial trophoblasts in both study groups, while the decrease in stromal cells was noted only in type-1 DM group. Beclin-1 expression was increased in interstitial and villous trophoblasts in both study groups. **Conclusion:** The expression of IGF-1, LIF, and Beclin-1 are altered in both the decidua and the trophoblasts in pregnancies of women with type-1 DM and MTHFR polymorphisms, compared to normal pregnancies undergoing (elective) terminations.

Abbreviations: DM: diabetes mellitus; E2: Estradiol; EPL: early pregnancy loss; Hcg: human chorionic gonadotropin; IGF-1: insulin-like growth factor 1; IL: interleukin; LIF: leukemia inhibitory factor; MFI: maternal-fetal interaction; MTHFR: methylenetetrahydrofolate reductase; NK: natural killer; TNF-α: tumor necrosis factor alpha

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MTHFR gene polymorphisms; type 1 diabetes mellitus; leukemia inhibitory factor; insulin like growth factor 1; beclin 1 protein

Introduction

Early pregnancy loss (EPL) is one of the main complications of pregnancy occurring in about 15% of pregnancies due to numerous etiological reasons [1–3]. The problem is not only the variety of etiologies, but also the lack of sufficient information about the

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pathophysiological changes at the site of maternal–fetal interaction (MFI), especially in the intervillous space of the placenta [4–6].

It has been reported that uncontrolled metabolic and immunological disorders may be associated with EPL [7–9]. Rates of EPL in women with type-1 diabetes mellitus (DM) are higher than those in women without carbohydrate metabolism disorders [8,10]. It has also been reported that EPL rates are higher in women with methylenetetrahydrofolate reductase (MTHFR) polymorphisms [11,12]. Possible reasons for the higher risk of these metabolic/immunological disorders are the toxic effects of hyperhomocysteinemia and hyperglycemia together with autoimmune antibodies and inflammatory cytokines in the cellular components of MFI [13–15].

Uncontrolled type-1 DM (hyperglycemia, presence of autoimmune antibodies, etc.) and hyperhomocysteinemia due to MTHFR polymorphisms are most probably responsible for the destruction of the cellular components of the intervillous space of the placenta (MFI) [16,17]. *The destruction of the cellular components of the MFI such as syncytiotrophoblasts, superficial epithelial cells of the decidua, endothelial cells of the spiral veins, and endovascular trophoblasts covering the tip of spiral arteries may cause inflammatory processes, which may result in perinatal/obstetric complications* [18,19]. The cell debris of syncytiotrophoblasts and endovascular trophoblasts, which contain paternal components, are especially important in the activation of maternal innate and humoral immune systems. Cell debris and inflammatory materials may also be responsible for the induction of *increased apoptotic activity of the syncytiotrophoblasts of the chorionic villi which can result in impaired villus/fetal perfusion by increased deposition of intervillous fibrin* [20–22]. Higher levels of apoptosis were also reported to be associated with impaired placentogenesis in various obstetric complications [23,24].

In this study, we assessed the expression of insulin-like growth factor 1 (IGF-1), leukemia inhibitory factor (LIF), and Beclin-1 markers to evaluate implantation/placentation. IGF-1 interacts with insulin to modulate its control of carbohydrate metabolism and it stimulates protein synthesis with a stimulatory influence on DNA synthesis and cell proliferation which is critical for implantation [25,26]. LIF is an interleukin (IL)-6 family cytokine that is involved in decidualization, implantation, blastocyst growth and development, and with the MFI [27,28]. Beclin-1 plays a role in the regulation of autophagy, apoptosis, and various cellular processes, and is critical during implantation [29,30].

This study was designed to determine whether markers (IGF-1, LIF, Beclin-1) of impaired implantation and decidualization are associated with metabolic (type-1 DM) and immunological (MTHFR polymorphisms) alterations.

Materials and methods

We used spontaneous abortion tissue samples of 7–11th gestational weeks (placental material + endometrium) from eight pregnant women with type-1 DM without MTHFR polymorphisms and eight pregnant women with MTHFR polymorphisms without type-1 DM (homozygous for MTHFR C677T). Additionally, elective abortion tissue samples with similar characteristics from eight normal pregnant women with neither type-1 DM nor MTHFR polymorphisms were used as a control group. Cases with abnormal fetal

karyotyping were excluded from the study. The obtained tissues (decidual, endometrial, placental) were fixed in 10% buffered formaldehyde, and samples were processed in a tissue processing machine under fixed vacuum (Leica, Germany).

Immunohistochemistry analysis of IGF- 1, LIF, and Beclin-1 in the tissue samples was evaluated by indirect ABC (avidin-biotin-peroxidase complex kit, Vectastain ABC kit, Vector Labs, Burlingame, CA) immunohistochemistry. Paraffin sections (3–4 μm thick) 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 antigen retrieval, sections were heated 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 (ABC Kit) for 20 min, LIF rabbit IgG antibody (1:200; Abbiotec, San Diego, Cat. No. 251429), IGF- 1 polyclonal antibody (1:100; MyBiosource, San Diego, Cat. No.MBS220960), and Beclin- 1 polyclonal antibody (1:100; MyBiosource, San Diego, Cat. No.MBS615615) were added at room temperature, and the sections were incubated for 1 h. In the negative control, PBS was added instead of primary antibody and the sections were incubated for 1 h. Sections were rinsed in PBS and incubated for 30 min with biotinylated secondary antibody (ABC Kit). After being rinsed in PBS, the sections were incubated for 30 min with ABC kit reagent (ABC Kit). Sites of peroxidase activity were visualized using DAB (Zymed, Cat. No. 00-2020) as a chromogen. The slides were counterstained with Mayer's hematoxylin.

The digital images were captured using the Leica DC 500 digital camera (Germany). Degree of immunoreactivity was noted as follows: (−): no immunoreactivity; (+): weak immunoreactivity; (++): moderate (mild) immunoreactivity; (+++): strong immunoreactivity (Staining; 0%: −; 1–30%: +; 31–60%: ++; 61–100%: +++). Each section was evaluated independently by two histologists blinded to the groups. The results obtained from the two histologists were compared and an average was determined. Five random regions of the MFI site were selected on each slide and examined at 400× magnification.

The frequency and percentage values were used for describing the qualitative data. For the quantitative data, the mean, median, standard deviation, and minimum and maximum values were reported as descriptive statistics. Normality assumption was assessed by Shapiro Wilk's test, and since the data did not conform to the normal distribution, Kruskal–Wallis analysis of variance was used for group comparisons. The Conover test was used for multiple comparisons following a significant Kruskal–Wallis test result. Friedman test was used for comparison of the surface epithelium, glandular epithelium, and decidual cells of the same patient. For all statistical analyses, the significance level was set at $p < 0.05$. The IBM SPSS for Windows version 21 statistical software was used.

Figure 1 shows the types of cells (superficial epithelial cells, glandular epithelial cells, stromal cells, decidual cells, endothelial cells, lymphocytes, interstitial trophoblasts, and villous trophoblasts) in the samples used during the course of the evaluation.

This study was approved by the Ethical Committee of Hacettepe University Faculty of Medicine, Ankara, Turkey (FON10/36). The consent forms were signed by all patients according to the regulations. The study has been carried out in accordance with The Code of Ethics of the World Medical Association.

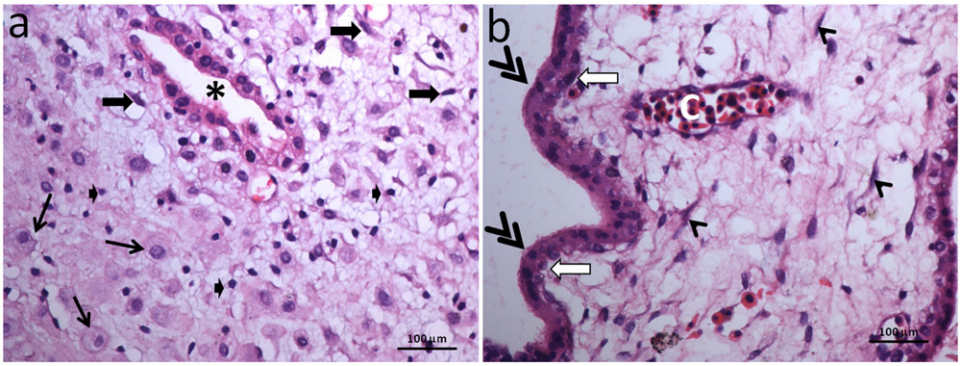

Figure 1. a: decidualized endometrium; endometrial gland (black asterick), decidual cells (thin, long black arrows), interstitial trophoblasts (thick, long black arrows), and lymphocytes (thick, short arrows); b: tertiary chorionic villi; cytotrophoblasts (black & white arrows), syncytiotrophoblasts (double black arrow head), fetal capillary (C) (a and b: Hematoxylin and eosin ×400).

Table 1. The expression of IGF-1, LIF, and Beclin-1 on the cell types (superficial epithelial cells, glandular epithelial cells, stromal cells, decidual cells, extravillous trophoblasts, endothelial cells, lymphocytes, vascular smooth muscle cells, cytotrophoblasts, syncytiotrophoblasts) used during the course of the evaluation.

		Control group		DM group		MTHFR group	
Cell	Marker	Cytoplasma	Nucleus	Cytoplasma	Nucleus	Cytoplasma	Nucleus
Superficial Epithelial Cells	LIF	+++	–	++	–	++	–
	IGF-1	+++	–	+	–	++	–
	Beclin-1	+++	–	++	–	++	–
Glandular Epithelial Cells	LIF	+++	–	++	–	++	–
	IGF-1	+++	–	+	–	++	–
	Beclin-1	+++	–	++	–	++	–
Stromal cells	LIF	++	–	++	–	++	–
	IGF-1	+++	–	++	–	+++	–
	Beclin-1	+++	–	++	–	+++	–
Decidual cells	LIF	++	–	+	+	++	–
	IGF-1	++	–	+	–	+	–
	Beclin-1	++	–	+	–	+++	–
Extravillous trophoblasts	LIF	++	–	++	–	+++	–
	IGF-1	+++	–	++	–	++	–
	Beclin-1	++	–	+++	–	+++	–
Endothelial cells	LIF	++	–	++	–	++	–
	IGF-1	+++	–	++	–	++	–
	Beclin-1	–	–	–	–	–	–
Lymphocytes	LIF	–	–	–	–	++	–
	IGF-1	–	–	–	–	–	–
	Beclin-1	+++	–	–	–	+++	–
Vascular smooth muscle cells	LIF	–	–	–	–	–	–
	IGF-1	++	–	–	–	+	–
	Beclin-1	–	–	–	–	–	–
Cytotrophoblasts	LIF	+++	–	+++	–	+++	–
	IGF-1	+	–	++	–	++	–
	Beclin-1	+	–	++	–	++	–
syncytiotrophoblasts	LIF	+++	–	+++	–	+++	–
	IGF-1	++	–	++	–	++	–
	Beclin-1	+	–	++	–	++	–

DM: Diabetes mellitus, MTHFR: methylenetetrahydrofolate reductase, LIF: Leukemia inhibitory factor, IGF-1: Insulin-like growth factor 1.

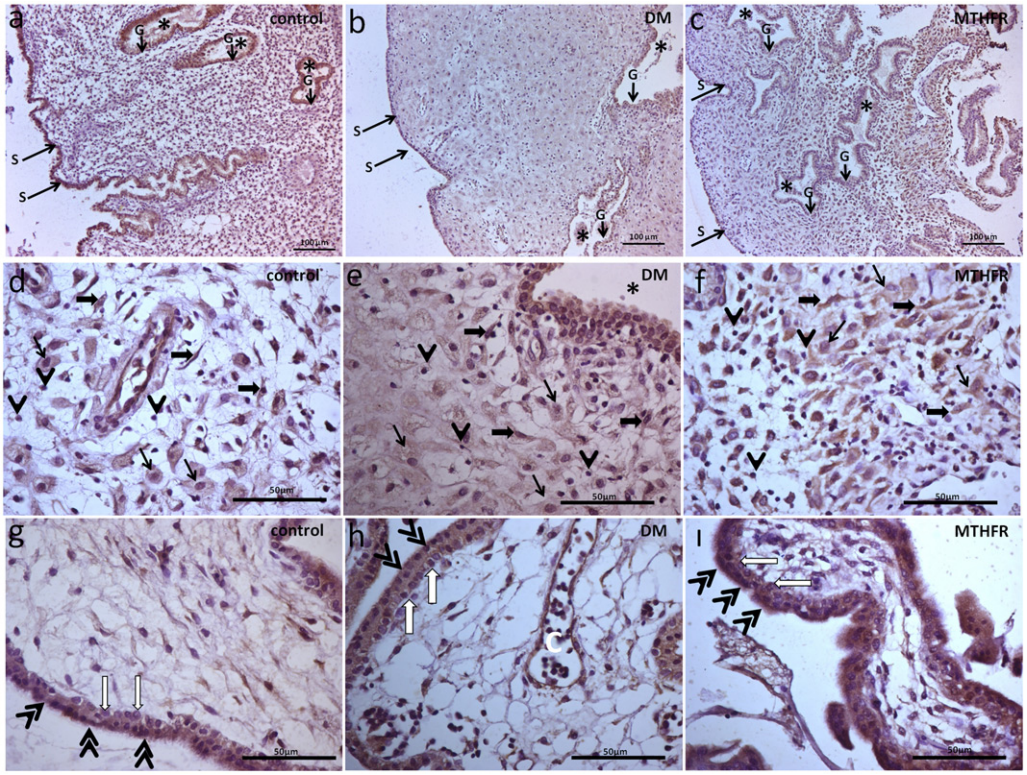

Figure 2. a–i: IGF-1 immunohistochemistry; a, d, g: control group; b, e, h: DM group; c, f, i: MTHFR group; a, b, c: endometrial tissue, superficial epithelial cells (S with black arrow), endometrial glands (black asterisk), glandular epithelial cells (G with black arrow); d, e, f: decidualized endometrium; endometrial gland (black asterisks), decidual cells (thin, long black arrows), interstitial trophoblasts (thick, long black arrows), and nonreactive lymphocytes (black arrow head); g, h, i: tertiary chorionic villi; cytotrophoblasts (black & white arrows) , syncytiotrophoblasts (double black arrow head), fetal capillary (C); (a, b, c: ABC Method-Hematoxylin ×100, d, e, f, g, h, i: ABC Method-Hematoxylin ×400).

Results

Table 1 shows the expression of IGF-1, LIF, and Beclin-1 on the cell types (superficial epithelial cells, glandular epithelial cells, stromal cells, decidual cells, extravillous trophoblasts, endothelial cells, lymphocytes, vascular smooth muscle cells, cytotrophoblasts, syncytiotrophoblasts) used during the course of the evaluation.

IGF-1 expression was shown to be decreased in the superficial and glandular cells of the decidua in both study groups compared to the control group, and it was more decreased in the type-1 DM group. IGF-1 expression in the decidual cells was decreased in both the study groups, while decrease in IGF-1 expression in the stromal cells was observed only in type-1 DM group (Fig. 2) Moreover, IGF-1 expression was decreased in the interstitial trophoblasts in both the MTHFR and type-1 DM groups, while it was increased in the villous cytotrophoblasts, especially, in the MTHFR group (Fig. 2).

The results showed that cytoplasmic LIF expression decreased in the superficial and glandular epithelial cells of the decidua in both MTHFR and type-1 DM groups (Fig. 3). Another significant finding was the presence of LIF expression in the lymphocytes in

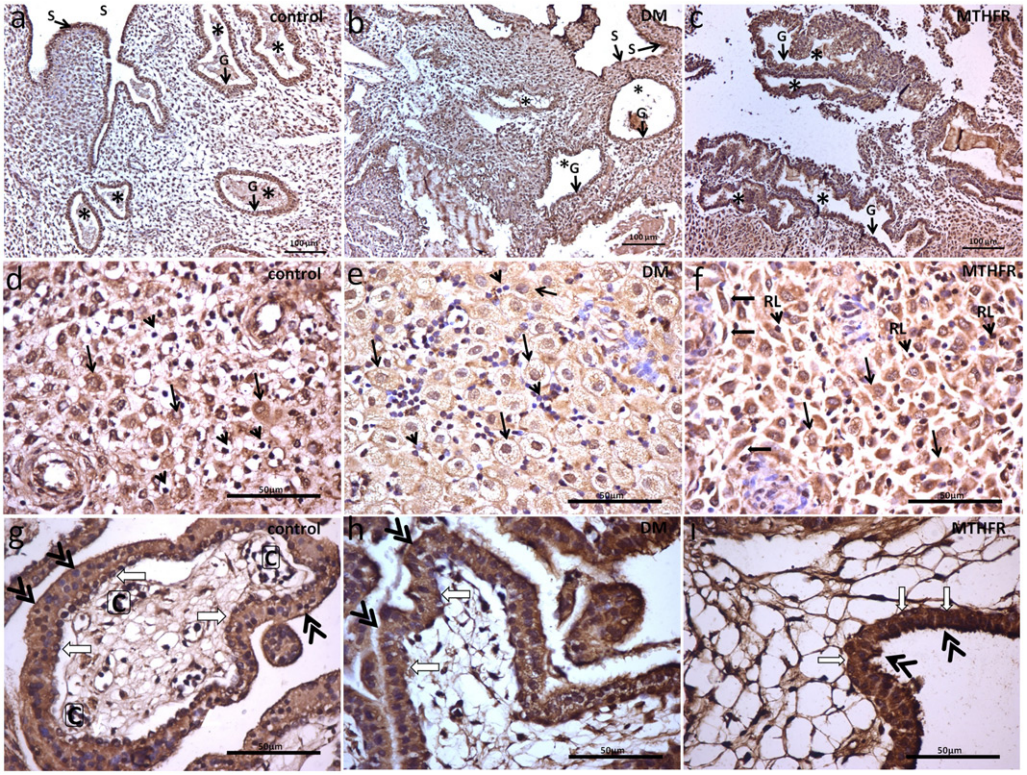

Figure 3. a–i: LIF immunohistochemistry; a, d, g: control group; b, e, h: DM group; c, f, i: MTHFR group; a, b, c: endometrial tissue, superficial epithelial cells (S with black arrow), endometrial glands (black asterisk), glandular epithelial cells (G with black arrow); d, e, f: decidualized endometrium; decidual cells (thin, long black arrows), interstitial trophoblasts (thick, long black arrows), nonreactive lymphocytes (black arrow head); immunoreactive lymphocytes (RL and black arrow head), vessel (v); g, h, i: tertiary chorionic villi; cytotrophoblasts (black & white arrows), syncytotrophoblasts (double black arrow head), fetal capillary (C); (a, b, c: ABC Method-Hematoxylin staining, 100× magnification; d, e, f, g, h, i: ABC Method-Hematoxylin staining, 400× magnification).

the MTHFR group as compared to that in the control and type-1 DM groups. LIF expression in the interstitial trophoblasts was also found to be increased in the MTHFR group compared to that in the other groups (Fig. 3).

Cytoplasmic Beclin-1 expression was reduced in the superficial and glandular epithelial cells of the decidua in both the MTHFR and type-1 DM groups (Fig. 4). Beclin-1 expression in the lymphocytes in the type-1 DM group was equivocal, while the expression of this marker was found to be increased in the interstitial and villous trophoblasts in both the study groups (Fig. 4).

Discussion

Type-1 DM and MTHFR polymorphisms are reportedly associated with increased perinatal morbidity/mortality and obstetrical complications, including higher rates of EPL [8,15]. Metabolic changes in these pathological conditions and the immunological changes in type-1 DM seem to be the biological rational behind the mechanisms

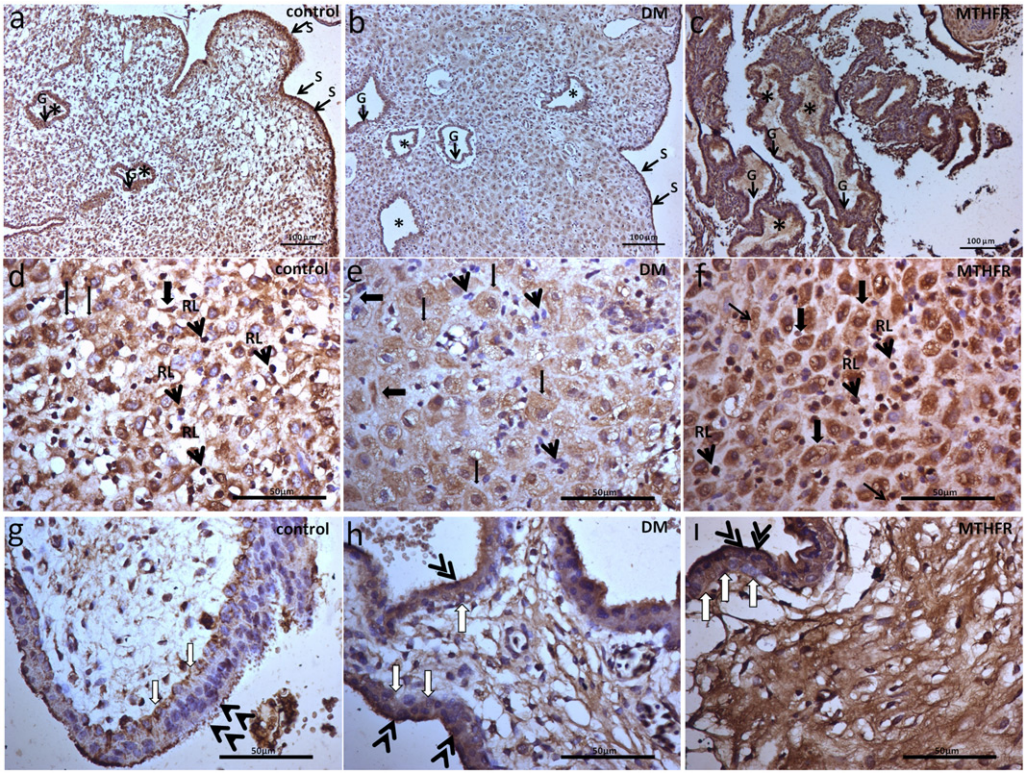

Figure 4. a–i: Beclin-1 immunohistochemistry; a, d, g: control group; b, e, h: DM group; c, f, i: MTHFR group; a, b, c: endometrial tissue, superficial epithelial cells (S with black arrow), endometrial glands (black asterisk), glandular epithelial cells (G with black arrow); d, e, f: decidualized endometrium; decidual cells (thin, long black arrows), interstitial trophoblasts (thick, long black arrows), nonreactive lymphocytes (black arrow head); immunoreactive lymphocytes (RL and black arrow head) g, h, i: tertiary chorionic villi; cytotrophoblasts (black & white arrows), syncytiotrophoblasts (double black arrow head), (a, b, c: ABC Method-Hematoxylin staining 100× magnification; d, e, f, g, h, i: ABC Method-Hematoxylin staining 400× magnification).

involved in EPL most probably by activating inflammatory processes in the placenta [16,17]. We hypothesize that hyperglycemia, hyperhomocysteinemia, and autoimmune antibodies may contribute to the destruction of the cellular components at the MIF, and the entrance of degenerative cell material into maternal circulation may trigger an inflammatory process [18–20]. In this study, we demonstrated that cellular structures of both the decidua and the trophoblasts of chorionic villi have alterations in IGF-1, LIF, and Beclin-1 expression in pregnancies with type-1 DM and MTHFR polymorphism.

We have shown that IGF-1, LIF, and Beclin-1 expression profile in the cellular components of intervillous space were different in pregnancies with metabolic and immunological inflammations of the placenta compared to those in normal pregnancies.

LIF, an implantation marker, is a 180-amino acid (40–50 kDA) glycoprotein belonging to the IL-6 family [27,31]. It has various receptors (LIF-R β and GP130) with different functions that are expressed on the surface of different cell types of the placenta [31,32].Various hormones and cytokines (HCG, E2, IGF 1 & 2, IL-1, TNF-α) induce LIF production, and placental natural killer (NK) cells express and control its expression

in the interstitial trophoblasts [32,33]. It activates decidualization, HCG secretion, and trophoblast proliferation [27,33]. In this study, we observed decreased LIF expression in the superficial and glandular epithelial cells of the decidua in MTHFR and type-1 DM groups. Furthermore, increased LIF expression in the trophoblasts and lymphocytes in the MTHFR group supports the hypothesis of impaired "decidualization/implantation" and trophoblastic migration [34].

IGF-1 (somatomedin-C/mechano growth factor) is a 7649 kDa single chain polypeptide (proinsulin analog) activating DNA synthesis and trophoblast proliferation [26]. It is controlled by growth factors, and it activates and supports decidualization [35]. It has six IGF-binding proteins [26]. In our study, IGF-1 expression was found to be reduced in the superficial and glandular cells of the decidua and in decidual cells in both MTHFR and type-1 DM groups. Its expression was also found to be reduced in the interstitial trophoblasts, while it was increased in the villous cytotrophoblasts which may indicate impaired placentation.

Beclin-1 plays a role in the early phase of autophagy and activates the formation of autophagic vesicles. It exists on the villous trophoblasts until the end of the second trimester and is effective during embryogenesis [29,30]. It has been reported that Beclin-1 gene mutations may be associated with early fetal death in mice [36]. In this study, cytoplasmic Beclin-1 expression was found to be reduced in the superficial and glandular epithelial cells of the decidua in both the study groups indicating reduced endometrial receptivity. We also demonstrated increased expression of this marker in interstitial and villous trophoblasts in both the clinical models. Lack of cytoplasmic expression in the lymphocytes in the type-1 DM group is still to be explained together with decreased and unclear expression of this marker in the decidual/stromal cells. Impaired endometrial receptivity (resistance to the graft) and affected trophoblastic migration seem to occur together with problematic fetal perfusion.

In conclusion, we demonstrated that the expression of IGF-1, LIF, and Beclin-1 are altered in both the decidua and the trophoblasts in pregnancies of women with type-1 DM and MTHFR polymorphisms, compared to normal pregnancies undergoing (elective) terminations. Future work including a spontaneous abortion group without type-1 DM or MTHFR is needed to determine if these changes at the MFI in pregnant women with either MTHFR polymorphisms or type-1 diabetes affect placentation. Furthermore, altered expression of IGF-1, LIF, and Beclin-1 could be markers of impaired placentation that may or may not be particular to type-1 DM or MTHFR, and additional studies would be necessary in order to investigate the significance of these findings.

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Disclosure statement

All the authors declare that there is no conflict of interest.

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References

[1] Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. Fertil Steril. 2003;79(3):577–84.
[2] Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. N Engl J Med. 1988;319(4):189–94. doi:10.1056/NEJM198807283190401.
[3] Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Best Prac Res Clin Obstet Gynaecol. 2000;14(5):839–54. doi:10.1053/beog.2000.0123.
[4] Cartwright JE, Whitley GS. Strategies for investigating the maternal-fetal interface in the first trimester of pregnancy: what can we learn about pathology? Placenta. 2017, 60: 145–149.
[5] Roberts JM, Redman CW, Collaboration GP. Global pregnancy collaboration symposium: prepregnancy and very early pregnancy antecedents of adverse pregnancy outcomes: overview and recommendations. Placenta. 2017, 60:103.
[6] Moffett A, Chazara O, Colucci F. Maternal allo-recognition of the fetus. Fertil Steril. 2017; 107(6):1269–72. doi:10.1016/j.fertnstert.2017.05.001.
[7] Xie X, Zhang Y, Xin L, Leng J, Lu Y, Xue Y. Relationship of folate metabolism related enzymes MTHFR and MTRR gene polymorphisms with unexplained recurrent spontaneous abortion. Int J Clin Exp Pathol. 2017;10(3):3746–52.
[8] Al-Agha R, Firth R, Byrne M, Murray S, Daly S, Foley M, Smith S, Kinsley B. Outcome of pregnancy in type 1 diabetes mellitus (T1DMP): results from combined diabetes-obstetrical clinics in Dublin in three university teaching hospitals (1995-2006). Ir J Med Sci. 2012;181(1):105–9. doi:10.1007/s11845-011-0781-6.
[9] Cervera R, Balasch J. Autoimmunity and recurrent pregnancy losses. Clin Rev Allergy Immunol. 2010;39(3):148–52. doi:10.1007/s12016-009-8179-1.
[10] Tennant PW, Glinianaia SV, Bilous RW, Rankin J, Bell R. Pre-existing diabetes, maternal glycated haemoglobin, and the risks of fetal and infant death: a population-based study. Diabetologia. 2014;57(2):285–94. doi:10.1007/s00125-013-3108-5.
[11] Yang Y, Luo Y, Yuan J, Tang Y, Xiong L, Xu M, Rao X, Liu H. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. Arch Gynecol Obstet. 2016;293(6): 1197–211. doi:10.1007/s00404-015-3944-2.
[12] Chen H, Yang X, Lu M. Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: a systematic review and meta-analysis. Arc Gynecol Obstet. 2016;293(2):283–90. doi:10.1007/s00404-015-3894-8.
[13] Zhou J, Ni X, Huang X, Yao J, He Q, Wang K, Duan T. Potential role of hyperglycemia in fetoplacental endothelial dysfunction in gestational diabetes mellitus. Cell Physiol Biochem. 2016;39(4):1317–28. doi:10.1159/000447836.
[14] Bergen N, Jaddoe V, Timmermans S, Hofman A, Lindemans J, Russcher H, Raat H, Steegers-Theunissen R, Steegers E. Homocysteine and folate concentrations in early

pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. BJOG. 2012;119(6):739–51. doi:10.1111/j.1471-0528.2012.03321.x.
[15] Bhatia N, Hemanshu B. Hyperhomocysteinemia in recurrent pregnancy loss. Int J Reprod Contracept Obstet Gynecol. 2017;6(7):2919–22. doi:10.18203/2320-1770.ijrcog20172907.
[16] Gauster M, Majali-Martinez A, Maninger S, Gutschi E, Greimel PH, Ivanisevic M, Djelmis J, Desoye G, Hiden U. Maternal type 1 diabetes activates stress response in early placenta. Placenta. 2017;50:110–6. doi:10.1016/j.placenta.2017.01.118.
[17] Kamudhamas A, Pang L, Smith SD, Sadovsky Y, Nelson DM. Homocysteine thiolactone induces apoptosis in cultured human trophoblasts: a mechanism for homocysteine-mediated placental dysfunction? Am J Obstet Gynecol. 2004;191(2):563–71. doi:10.1016/j.ajog.2004.01.037.
[18] Mumusoglu S, Beksac MS, Ekiz A, Ozdemir P, Hascelik G. Does the presence of autoantibodies without autoimmune diseases and hereditary thrombophilia have an effect on recurrent pregnancy loss? J Matern Fetal Neonatal Med. 2016;29(14):2352–7. doi:10.3109/14767058.2015.1085964.
[19] Beksaç K, Örgül G, Çagan M, Karaagaoglu E, Arslan S, Beksaç MS. Retrospective evaluation of pregnant women with celiac disease. J Turk Ger Gynecol Assoc. 2017;18(1):56. doi:10.4274/jtgga.2016.0198.
[20] Triggianese P, Perricone C, Chimenti MS, De Carolis C, Perricone R. Innate immune system at the maternal-fetal interface: mechanisms of disease and targets of therapy in pregnancy syndromes. Am J Reprod Immunol. 2016;76(4):245–57. doi:10.1111/aji.12509.
[21] Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol. 2017;17(8):469–82. doi:10.1038/nri.2017.64.
[22] Xu Y-Y, Wang S-C, Li D-J, Du M-R. Co-signaling molecules in maternal–fetal immunity. Trends Mol Med. 2017;23(1):46–58. doi:10.1016/j.molmed.2016.11.001.
[23] Langbein M, Strick R, Strissel PL, Vogt N, Parsch H, Beckmann MW, Schild RL. Impaired cytotrophoblast cell–cell fusion is associated with reduced Syncytin and increased apoptosis in patients with placental dysfunction. Mol Reprod Dev. 2008;75(1):175–83. doi:10.1002/mrd.20729.
[24] Christiansen OB, Nielsen HS, Kolte AM, editors. Inflammation and miscarriage. Seminars in fetal and neonatal medicine. WB Saunders, 2006. Vol. 11. No. 5. WB p. 302–308.
[25] Luo L, Wang Q, Chen M, Yuan G, Wang Z, Zhou C. IGF-1 and IGFBP-1 in peripheral blood and decidua of early miscarriages with euploid embryos: comparison between women with and without PCOS. Gynecol Endocrinol. 2016;32(7):538–42. doi:10.3109/09513590.2016.1138459.
[26] Chi MM-Y, Schlein AL, Moley KH. High insulin-like growth factor 1 (IGF-1) and insulin concentrations trigger apoptosis in the mouse blastocyst via down-regulation of the IGF-1 receptor. Endocrinology. 2000;141(12):4784–92. doi:10.1210/endo.141.12.7816.
[27] Rosario GX, Stewart CL. The multifaceted actions of leukaemia inhibitory factor in mediating uterine receptivity and embryo implantation. Am J Reprod Immunol. 2016;75(3):246–55. doi:10.1111/aji.12474.
[28] Cheng J, Rosario G, Cohen TV, Hu J, Stewart CL. Tissue-specific ablation of the LIF receptor in the murine uterine epithelium results in implantation failure. Endocrinology. 2017;158(6):1916–28. doi:10.1210/en.2017-00103.
[29] Cao B, Camden AJ, Parnell LA, Mysorekar IU. Autophagy regulation of physiological and pathological processes in the female reproductive tract. Am J Reprod Immunol. 2017; 77: e12650.
[30] Nakashima A, Aoki A, Kusabiraki T, Shima T, Yoshino O, Cheng SB, Sharma S, Saito S. Role of autophagy in oocytogenesis, embryogenesis, implantation, and pathophysiology of pre-eclampsia. J Obstet Gynaecol Res. 2017;43(4):633–43. doi:10.1111/jog.13292.
[31] Nicola NA, Babon JJ. Leukemia inhibitory factor (LIF). Cytokine Growth Factor Rev. 2015;26(5):533–44. doi:10.1016/j.cytogfr.2015.07.001.
[32] Yue X, Wu L, Hu W. The regulation of leukemia inhibitory factor. Cancer Cell Microenviron. 2015;2(3):e877. doi:10.14800/ccm.877

[33] Aghajanova L. Leukemia inhibitory factor and human embryo implantation. Ann N Y Acad Sci. 2004;1034(1):176–83. doi:10.1196/annals.1335.020.
[34] Hekimoğlu R, Pergin A, Uğur Y, Beksaç S, Turğal M, Çakar N. Impaired implantation and hereditary thrombophila; expression of LIF (leukemia inhibitory factor) on extravillous trophoblasts. Gynecol Obstet Reprod Med. 2012;18:123–126.
[35] Kobayashi R, Terakawa J, Omatsu T, Hengjan Y, Mizutani T, Ohmori Y, Hondo E. The window of implantation is closed by estrogen via insulin-like growth factor 1 pathway. J Reprod Infertil. 2017;18(2):231–41.
[36] Tsukamoto S, Kuma A, Murakami M, Kishi C, Yamamoto A, Mizushima N. Autophagy is essential for preimplantation development of mouse embryos. Science. 2008;321(5885):117–20. doi:10.1126/science.1154822.