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# Expression of PECAM-1 and E-Cadherin in the Umblical Cords of Gestational Diabetic Mothers

## Expresión de PECAM-1 y E-cadherina en los Cordones Umbilicales de Madres con Diabetes Gestacional

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**SUMMARY:** The purpose of this study is to examine the changes in the umbilical cord in women diagnosed with gestational diabetes mellitus In this study, as a control group human placental tissues from normotensive pregnancies was collected from diabetic women at 28–35 weeks of gestation. Gestational diabetes (n= 20) and normal umbilical cord (n= 20) for a total of 40 units were received.GDM groups compared to the control group was significantly higher values was detected ($p<0.01$). In GDM group, light microscopy showed erosion of the endothelium and complete rupture of the umbilical vessels resulting in extravasation of blood within Wharton's jelly. it was observed that the cytoplasmic fragments and cell infiltration of the spill to the subepithelial layer of apoptotic cell PECAM-1 positive reaction showed. E-Cadherin in endothelial side surface of diabetes group showed weak expression in the nucleus and showed positive reaction in smooth muscle.

**KEY WORDS: Gestational diabetes mellitus; PECAM-1; Umblical cord; E-Cadherin.**

## INTRODUCTION

Gestational diabetes mellitus(GDM) patients have an increased risk of co-morbidities during pregnancy, e.g. preeclampsia, pregnancy-induced hypertension with impeded delivery (Fadl *et al*., 2010). GDM is one of the common metabolic diseases during pregnancy, affecting up to about 14% of all pregnancies. It is becoming more prevalent due to the characteristics of modern living, such as an advanced maternal age at first pregnancy and changes in dietary habits (Ferrara, 2007). The umbilical cord provides a functional connection between the fetus and the placenta, its importance for the development of the fetus is unquestionable. Gestational diabetes (GDM) is associated with increased oxidative stress and overexpression of inflammatory cytokines, both of which might lead to endothelial dysfunction and vascular disease (Di Fulvio *et al*., 2014). the intra-uterine environment consisting of hyperglycemia, hyperinsulinemia and insulin resistance are associated with endothelial dysfunction in their offspring (Leiva *et al*., 2011).

The umblical cord is covered by an epithelium derived from the enveloping amnion. The network of glycoprotein microfibrils and collagen fibrils in the Wharton's jelly has been previously studied (Meyr *et al*., 1983).The interlaced collagen fibers and small, woven bundles are arranged to form a continuous soft skeleton that encases the umbilical vessels (Vizza *et al*., 1996).

PECAM-1 is constitutively expressed in all vessel types, localizing to areas of cell–cell junctions in addition to lumen-facing areas of blood vessels. PECAM-1 has been shown to play an important role in the adhesion cascades leading to extravasation of leukocytes during inflammation in vitro (Muller *et al*., 1993) and in vivo (Vaporciyan *et al*., 1993; Duncan *et al*., 1999; Liao *et al*., 1999) The purpose of this study is to examine the changes in the umbilical cord in women diagnosed with gestational diabetes mellitus.

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## MATERIAL AND METHOD

The study protocol was approved by the Medical Committee of Diyarbakir Hospital Maternity and Child Health Hospital and informed consent was obtained from all subjects involved in the study. In this study, the umbilical cord was obtained from a total of 40 pregnant women attended at the department of obstetrics and gynecology clinic of Diyarbakir Maternity and Children Hospital. Twenty healthy patients of the same age with HELLP syndrome were included in the sample.

Umbilical cords of babies born at 28–38 weeks of pregnancy were removed. Gestational diabetes (n= 20) and normal umbilical cords (n= 20) for a total of 40 units were received. Venous blood samples were taken 15 minutes after patients completed a 12 hour fast (between 08:00 and 10:30 am. Gestational diabetes mellitus was diagnosed by 50 g OGTT (oral glucose tolerance test) venous plasma / serum threshold values as follows: 50 g OGTT <140 mg/dL is normal; 50 g OGTT 140–200 mg/dL is a 3-h 100 g OGTT. 50 g OGTT ≥200 mg/dL in patients GDM is diagnosed directly and treatment begins. Fasting blood glucose ≥140 mg/dL are considered diabetes in this case

Each umbilical cord was immediately clamped at delivery. In all cases, 1–12 cm-long sections of umbilical cord were cut, The specimens were immersed in 10% buffered formaldehyde. They were dehydrated in a graded ethanol series, cleaned in xylene and embeded in parafin, then 4 µm sections were cut and made into slides. These were stained with hematoxylene Eosin and Trichrom Masson.

**Immunohistochemical Technique**. Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized and rinsed in absolute alcohol. Antigen retrieval process was performed twice in citrate buffer solution (pH: 6.0); the first for 7 min, and the second for 5 min boiled in a microwave owen at 700 W. They were allowed to cool to room temperature for 30 min and washed twice in distilled water for 5 min. Endogenous peroxidase activity was blocked in 0.1% hydrogen peroxide for 20 min. Ultra V block (Cat.No:85-9043, Invitrogen, Carlsbad, CA,USA) was applied for 10 min prior to the application of primary antibodies (E-cadherin antibody, Santa-Cruz 1:100) and (mouse monoclonal anti-PECAM-1(CD31); Santa-Cruz) 1:100 overnight. Secondary antibody (Cat.No:85- 9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 min. Slides were then exposed to streptavidin–peroxidase for 20 min. Chromogen, diaminobenzidine (DAB Invitrogen, Carlsbad, CA, USA) was used. Control slides were prepared as mentioned above but omitting the primary antibodies. After counterstaining with hematoxylin, washing in tap water for 8 minutes and in distilled water for 10 min, the slides were mounted with entellan.

**Statistical Analysis.** Statistical analyses of the data were conducted by using Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL). Data were presented as Mean ± Standard deviation. Normality was evaluated with the Kolmogorov-Smirnov test. Variables with non-parametric distribution were compared using the Mann-Whitney U test. A P value less than 0.05 was accepted as statistically significant. Morphometric examination, endothelial core length, among all the parameters were significantly different between the groups.

## RESULT AND DISCUSSION

In GDM group, light microscopy showed erosion of the endothelium and complete rupture of the umbilical vessels resulting in extravasation of blood within Wharton's jelly. The umbilical vein was grossly dilated and thin walled. Smooth muscles of the umbilical vessel showed disruption and degeneration of its fibers (Fig. 1).

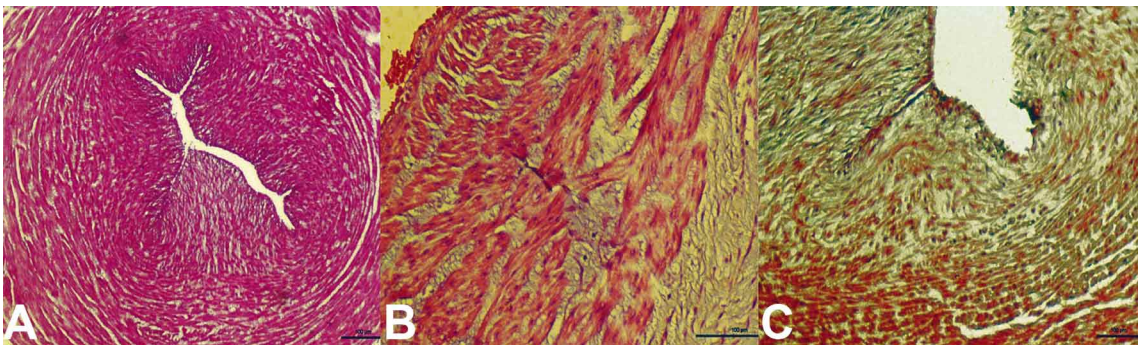


Fig. 1. A)Control group. Normal appearance of umbilical cord (H-E stain, Bar 100 µm), b and c) GDM groups mononuclear cell infiltration in subendothelial layer, leaving the smooth muscle bundles, connective tissue growth and showed thinning of the collagen fibers in the wharton gel (B: H-E stain, Bar 50 µm; C: Trichrom masson stain, Bar 100 µm).

Gestational diabetes mellitus imposes a great challenge for gynecologists due to increased risk of the mother and the child developing overt diabetes and associated vascular complications (Kim *et al*., 2002; Carr *et al*., 2006; Räsänen & Kirkinen, 1983). Diabetic insult at later stages in gestation, such as in GDM, will lead to short-term changes in a variety of molecules for key functions including gene expression (Mayhew, 2002). The relationship between umbilical cord morphology and prenatal complications such as intrauterine losses, gestational diabetes, preeclampsia, intrauterine growth retardation and fetal distress has emphasized by some researchers (Raio *et al*., 1999; Di Naro *et al*., 2001a; Weissman & Jakobi, 1997; Goodlin, 1987; Silver *et al*., 1987). Presence of high glucose in GDM subjects may be involved in endothelial inflammation, as there are reports of association of high maternal glucose with complications like Chorioamnionitis, an inflammation of the fetal membranes (Scholl *et al*., 2001; Luke *et al*., 2005). Obesity is one of the major causes of gestational diabetes and endothelial inflammation (Denison *et al*., 2010). Weissman & Jakobi found that umbilical cord diameter was significantly larger in pregnancies complicated by gestational diabetes mellitus. Wharton's jelly is a soft connective tissue and consists mainly of fibroblasts and macrophages which are embedded in a homogeneous jelly like intercellular substance (Di Naro *et al*., 2001b). The Wharton's jelly acts as a protective barrier on the wall structure of the vein and arteries. In our study, diabetes depending on the collagen fibers in the Wharton's Jelly separation and observed an increase in the intermediate. the umbilical cord, the collagen fibers can be considered to affect this barrier. PECAM-1 has been shown to be a key player in the adhesion cascade leading to extravasation of leukocytes during inflammation (Muller *et al*.). In our study, it was observed that the cytoplasmic fragments and cell infiltration of the spill to the subepithelial layer of apoptotic cell PECAM-1 positive reaction showed (Fig. 2). PECAM-1, the adjacent adhesion between endothelial cells and it is considered that if the interactions between the immune cells; GDM result of this adhesion can be prevented, E-Cadherin in endothelial side surface of diabetes group showed weak expression in the nucleus and showed positive reaction in smooth muscle. E-Cadherin cell separation and connection can play an important role in separation of the complex.

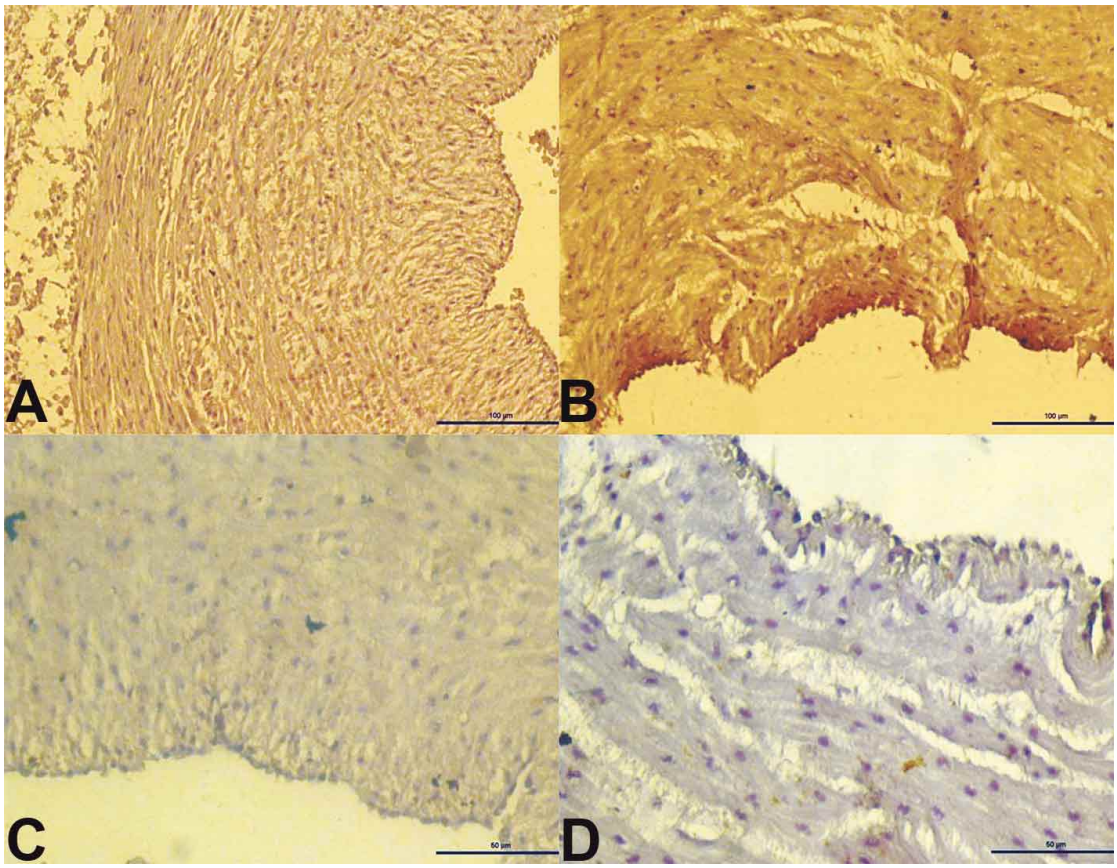


Fig. 2. A) Control group (PECAM-1 immunostaining, Bar 100 μm). B) GDM group PECAM-1 shedding and appearance of a truncated cytoplasmic fragment during endothelial cell apoptosis (PECAM-1 immunostaining, Bar 50 μm). C) Control group (E-Cadherin immunostaining, Bar 50 μm). D) GDM group, weak E-Cadherin expression in endothelial side surface, positive expression in endothelial nucleus and smooth muscle (E-Cadherin immunostaining, Bar 50 μm).

Table I. Morphometric parameters in both groups.

| Group | | Gestasyonal diabetes (n= 20) | Control (n= 20) | P Value* |
|---|---|---|---|---|
| **Arterial wall thickness** | Mean (SD) | 102.3 (8.06) | 81.0 (7.73) | <0.001 |
| | Median (SEM) | 98.9 (2.54) | 80.2 (2.44) | |
| | Range | 84.7–102 | 66.3–81.3 | |
| **Vein wall thickness** | Mean (SD) | 88.3 (8.84) | 73.1 (11.0) | 0.008 |
| | Median (SEM) | 90. (2.79) | 69.9 (3.20) | |
| | Range | 63.8–88.9 | 45.3–73.3 | |
| **Basement membrane thickness** | Mean (SD) | 9.07 (2.41) | 4.33 (0.71) | <0.001 |
| | Median (SEM) | 8.40 (0.76) | 4.73 (0.22) | |
| | Range | 6.26–14.7 | 3.52–5.63 | |
| **Amnion cell nucleus length** | Mean (SD) | 8.14 (2.02) | 4.34 (1.34) | 0.001 |
| | Median (SEM) | 8.41 (0.63) | 4.18 (0.42) | |
| | Range | 4.25–11.7 | 3.02–7.37 | |
| **Endothelial cell nucleus length** | Mean (SD) | 7.27 (1.38) | 6.16 (1.54) | 0.092 |
| | Median (SEM) | 7.67 (0.43) | 6.22 (0.48) | |
| | Range | 4.70–8.80 | 3.40–8.57 | |
| **Fibroblast cell nucleus length** | Mean (SD) | 10.14 (0.77) | 6.37 (1.08) | <0.001 |
| | Median (SEM) | 9.96 (0.24) | 6.34 (0.43) | |
| | Range | 9.06–11.6 | 4.25–7.78 | |
| **Smooth muscle cell nucleus length** | Mean (SD) | 13.3 (2.02) | 9.62 (1.47) | 0.002 |
| | Median (SEM) | 13.6 (0.63) | 9.34 (0.46) | |
| | Range | 10.51–15.4 | 6.70–10.7 | |

* Mann–Whitney U Test.

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**RESUMEN:** El objetivo fue examinar los cambios que presenta el cordón umbilical de mujeres con diagnóstico de diabetes mellitus gestacional (DMG). Se incluyeron en el grupo control muestras de tejidos placentarios humanos de embarazos normotensos y de mujeres diabéticas de entre 28–35 semanas de gestación. Las muestras se divieron en cordones umbilicales con cambios de DMG (n= 20) y cordones umbilicales normales (n= 20), constituyendo un total de 40 muestras. El grupo de DMG, en comparación con el grupo control, presentó valores significativamente más elevados (p<0,01). En el grupo de DMG, la microscopía óptica demostró la erosión del endotelio y la ruptura completa de los vasos umbilicales, resultando en la extravasación de sangre dentro de la gelatina . Se observaron fragmentos citoplasmáticos e infiltración celular de la capa subepitelial de células apoptóticas mostró una reacción positiva a PECAM-1. En el grupo de DMG, la E-cadherina de la superficie lateral endotelial mostró una expresión débil en el núcleo y una reacción positiva en el músculo liso.

**PALABRAS CLAVE: Diabetes mellitus gestacional; PECAM-1; Cordón umbilical; E-cadherina.**

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