The Subendothelial Content of Fibronectin and Collagen Type IV in the Umbilical Vessels of Gestational Diabetic Mothers

GESTASYONEL DİABETİ OLAN ANNELERDEKİ UMBİLİKAL DAMARLARIN SUBENDOTELYAL FİBRONEKTİN VE KOLLAGEN TİP IV İÇERİĞİ

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Summary

Objective: *To investigate the subendothelial changes of fibronectin and collagen type IV in the umbilical vessels of diabetic mothers.*

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Materials and Methods: *Ten pregnant women with gestational diabetes confirmed by 100 g oral glucose tolerance test at term comprised the study group and ten healthy pregnant women with normal pregnancy at term comprised the control group. Quantitative and qualitative changes of collagen type IV and fibronectin distribution in the subendothelial region of umbilical arteries and veins of the two groups were investigated by immunohistochemical staining.*

Results: *Fibronectin content of umbilical vein in the control group was found to be significantly lower than the study group(P=0.039).*

Conclusion: *Subendothelial fibronectin content of the umbilical vein is found to be significantly higher in the gestational diabetic mothers.*

Key Words: Gestational diabetes mellitus, Fibronectin, Collagen type IV

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Özet

Amaç: *Diabetik annelerde umbilikal damarlardaki subendotelyal fibronektin ve tip IV kollagen değişiminin incelenmesi*

Çalışmanın Yapıldığı Yer: *Ege Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Kliniği, İZMİR*

Gereç ve Yöntem: *100 g oral glukoz tolerans testi ile gestasyonel diabet tanısı konmuş termde 10 gebe çalışma grubu ve sağlıklı termde 10 gebe kontrol grubu olarak alındı. Umbilikal arter ve venlerin subendotelyal kısımlarındaki tip IV kollagen ve fibronektin dağılımındaki değişiklikler kalitatif ve kantitatif olarak immunohistokimyasal boyama ile değerlendirildi.*

Bulgular: *Kontrol grubundaki fibronektin içeriği çalışma grubundan anlamlı olarak düşük bulundu (p=0.039)*

Sonuç: *Umbilikal venlerdeki subendotelyal fibronektin içeriği gestasyonel diabeti olan annelerde anlamlı olarak yüksek bulundu.*

Anahtar Kelimeler: Gestasyonel diabetes mellitus, Fibronektin, Kollagen tip IV

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Congenital malformations occur with increased frequency in the offspring of diabetic mothers and represent the major cause of perinatal mortality in these infants. Several studies have shown an association between poor metabolic control in the first trimester and major infant malformations (1,2). Although good metabolic control in the periconceptional period and through pregnancy may not provide complete prevention, it substantially reduces the incidence of congenital defects suggest-

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ing that the abnormal metabolic milieu during organogenesis is responsible for the increased malformation rate (3).

Extracellular matrix components (ECM) play an essential role in basement membrane organisation (2). These molecules control gene expression by modulating cell-cell and cell-matrix interaction and control gene expression. In developing embryos, ECM components have been shown to regulate cell migration, adhesion, proliferation and differentiation (4). Abnormalities of basement membranes, the specialized ECM situated at the boundary between cells and the underlying connective tissue, are a generalized phenomenon in diabetes mellitus. Increased synthesis of basement membrane collagen occurs in the retina and kidney of diabetic animals (5,6) and the fibronectin transcript is overexpressed in the heart and kidney of diabetic rats (7).

In this study subendothelial changes of collagen type IV and fibronectin provoked by high glucose or insulin levels in the umbilical arteries of diabetic mothers was investigated.

Materials and Methods

The study was performed starting from January 1997 through October 1998 in Ege University Faculty of Medicine, Obstetrics and Gynecology Department. The control group comprised of 10 pregnant patients at term without a history of glucose intolerance during their pregnancy. The study group comprised of 10 pregnant patients at term with gestational diabetes mellitus confirmed by 100 g oral glucose tolerance test. Five of the patients in the study group received diet alone and 5 of them diet and insulin therapy during their pregnancy. Exclusion criteria included the presence of hypertension, cigarette smoking and alcohol intake. Within 10 minutes after the delivery perfusion fixation of the umbilical artery and the vein was performed at a low pressure using buffered formalin at pH 7.2. All umbilical cord specimens were cut 10 cm away from the placenta, fixed immediately in 10% neutral formalin and embedded in paraffin. Five micrometer thick serial sections were cut from each block after routine microscopic laboratory technics and stained with the Haematoxylin-Eosin. Sections from formalin-fixed paraffin embedded tissue samples were taken to gelatine-coated slides and were treated in microwave oven according to Shi's method in order to establish a better antigenic determinant expression (5). Then the slides were incubated with rabbit anti-human fibronectin antiserum (DAKO, Germany) at 1:200 dilution for one hour and with prediluted anti-human collagen type IV antiserum (DAKO, Germany) for one hour. Immunohistochemical staining was performed by LSAB 2KIT (DAKO, Germany). All the slides were studied by two of the authors (C.S. and A.V.) without knowledge of the clinical data. Quantitative alterations of collagen type IV and fibronectin distribution in the subendothelial region of umbilical arteries and veins of the two groups were investigated at the light microscopic level by using the Optimas Programme Version 6.2 for Image Analysis (Figure 1, 2 and 3) by measuring the mean thickness of the immunostained area expressed in micrometers. The results were submitted to statistical analysis with the use of Students t-test for independent groups accepting $p< 0.05$ as significant.

Results

The clinical data of the patients were demonstrated in Table 1. The quantitative results were shown in Table 2 and Figure 1. Fibronectin content of umbilical vein in the control group was found to be significantly lower than the study group (P=0.039). The mean thicknesses of the basement

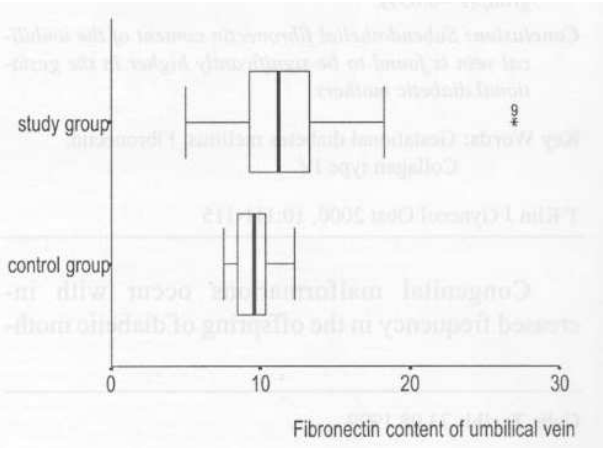

Figure 1. The difference between the fibronectin content of umbilical vein of the control and study groups as expressed by the mean thicknesses of the basement membranes.

Figure 2. Umbilical artery of the control group IHC staining with fibronectin 200x

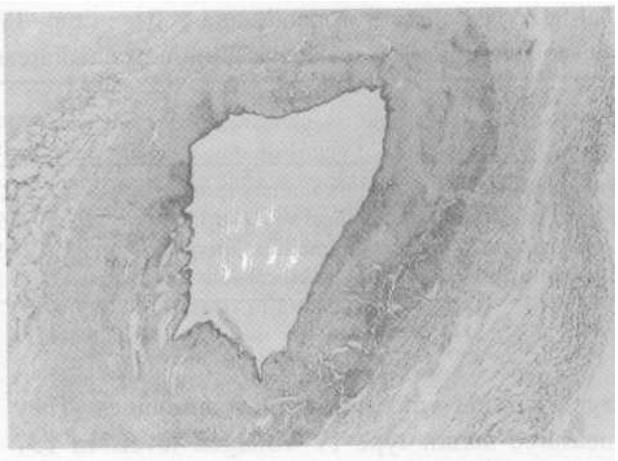

Figure 3. Umbilical vein the control group IHC staining with collagen type IV 100x

membranes immunostained by anti-human fibronectin antiserum in the control and study groups for the umbilical artery were 13.26±8.36 mm and 14.22±9.91 mm, respectively (p=0.638). The mean thicknesses of the basement membranes immunostained by anti-human fibronectin antiserum in the control and study groups for the umbilical vein were 9.63±1.49 mm and 12.53±6.16 mm, respectively (p=0.039). The mean thicknesses of the basement membranes immunostained by anti-human collagen type IV antiserum in the control and study groups for the umbilical artery were 21.91±7.31 mm and 16.13±9.61 mm, respectively (p=0.289). The mean thicknesses of the basement membranes immunostained by anti-human collagen type IV antiserum in the control and study groups for the umbilical vein were 19.36±6.41 mm and 17.85±5.35 mm, respectively (p=0.443).

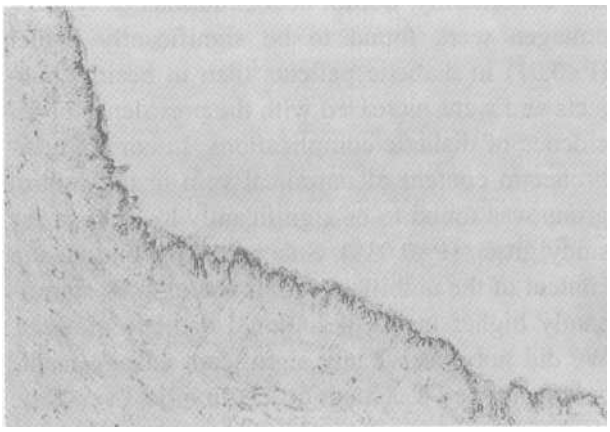

Figure 4. Umbilical vein of the gestational diabetic group IHC staining with fibronectin 200x

Table 1. Ages and placental weights of control and the study groups

	control group (n=10)	study group (n=10)
Mean placental weight (gram)	604±33	603±30
age (years)	27.6±3.7	26.3±3.9

Discussion

The diabetic pregnancy is characterised by numerous disturbances in fetal development. Both fetal macrosomia and intrauterine growth retardation are commonly seen in poorly controlled diabetes, the latter particularly in long-standing severe diabetes and in diabetes associated with vascular complications. Congenital malformations are also more frequent in diabetic pregnancies. The placenta in pregnancies complicated by diabetes is generally larger than normal and has numerous associated structural abnormalities that are likely to have a role in the resulting disturbances of fetal growth in diabetic pregnancy (8).

Matsumato et al (9) developed a sandwich enzyme immunoassay for human serum type IV col-

Table 2. The mean fibronectin and collagen type IV contents of umbilical artery and the vein as expressed by the mean thicknesses of the immunostained areas in the basement membranes (μm: micrometer)

	control group (n=10)	study group (n=10)	P value
Fibronectin content of umbilical artery (μm)	13.26±8.3 6	14.22±9.91	0.638
Fibronectin content of umbilical vein (μm)	9.63±1.49	12.53±6.16	0.039
Collagen type IV content of umbilical artery (μm)	21.91±7.31	16.13±9.61	0.289
Collagen type IV content of umbilical vein (μm)	19.36±6.41	17.85±5.35	0.443

lagen peptide with monoclonal antibodies. They measured serum type IV collagen levels in 137 non-insulin dependent diabetic patients (aged 50-75 year) with or without clinical signs of retinopathy, nephropathy and/or neuropathy and 110 healthy subjects (aged 50-75 year) without serological abnormality. Serum concentrations of type IV collagen were found to be significantly higher ($P<0.01$) in diabetic patients than in healthy subjects and were increased with the prevalence or incidence of diabetic complications. In our study fibronectin content of umbilical vein in the control group was found to be significantly lower than the study group ($P=0.039$). Subendothelial fibronectin content of the umbilical vein is found to be significantly higher in the gestational diabetic mothers. We did not observe any significant change in the collagen type IV content of the umbilical vessels.

Stoz et al (10) examined morphometrically the terminal villi of 26 patients with gestational diabetes in order to determine if there is an immaturity of placental development. Investigation of villous surface, degree of vascularization, and development of epithelial plates yielded values lying somewhere between those of non-diabetic patients and those of patients with overt diabetes. Only the surface areas of the vessels were reduced to levels lower than in overt diabetes.

Abnormal metabolic milieu of diabetes induces increased synthesis of basement membrane components, and these molecules play a prominent role in morphogenesis. Cagliero et al (3) investigated whether maternal diabetes or high glucose levels disturb extracellular matrix synthesis in rat embriyos. In gestational day 11 embryos, maternal diabetes induced a small but significant increase in laminin B1 (127±40% of control, $P<0.02$) but not in fibronectin mRNA (101±26% of control). Day 12 embryos from diabetic mothers showed a larger increment in laminin B1 (179±91% of control, $p<0.02$) and also an increase in fibronectin mRNA (172±73% of control, $P<0.02$). The finding that maternal diabetes induces increased expression of ECM in developing embriyos establishes a link with the abnormalities occurring in the chronic complications of diabetes.

In the present study, formalin-fixed umbilical cords were processed for immunoperoxidase staining with antibodies against fibronectin and collagen type IV. Intensity of immunostaining (immunoperoxidase reaction product) was attempted to quantify by image analysis. However, quantification of enzyme reaction is not quite accurate under these conditions (end point measurements at possible plateau phase). Moreover, immunohistocytochemical control experiments were not performed to confirm specificity of immunostaining. We conclude that gestational diabetes affects the basement membrane structure of umbilical vessels. This must be confirmed by other clinicopathologic studies with specific immunostaining including the effects on other collagen types.

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