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Relation of Endothelin-1 to Abnormal Umbilical Doppler Waveform Studies In Pregnancies Complicated By IDDM and Its Relation to the Neonatal Outcome

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Endothelin -1 appears to be the predominant member of the family (endothelins ET-1, ET-2 and ET-3), which is exclusively produced by vascular endothelial cells and has the most potent vasoconstrictor activity of all naturally occurring press or substances. Our objective is to evaluate whether Endothelin -1 has a role in altered umbilical arterial blood flow velocity in pregnant mothers with IDDM and its relation to neonatal outcome. 60 pregnant women were recruited for this study and were divided into two groups: 30 IDDM pregnant women with abnormal umbilical Doppler flow velocity waveforms and 30 IDDM pregnant women with normal umbilical artery flow velocity waveform studies. Levels of glycosylated hemoglobin and plasma concentration of ET-1 were determined. Neonatal assessment using the 5 minutes Apgar score and birth weight were estimated; also, plasma level of ET-1 was done for all neonates. A highly significant statistical difference of ET-1 levels between newborns of diabetics with abnormal flow velocity Doppler waveforms and newborns of diabetics with normal Doppler waveforms ($P<0.001$). In addition, there was a highly significant statistical difference of Apgar score and birth weights between newborns of diabetics with abnormal flow velocity Doppler waveform and newborns of diabetics with normal Doppler waveform. We can conclude that although IDDM pregnant women have markedly elevated ET-1 levels, it is not responsible for the vascular changes associated with abnormal umbilical Doppler studies. Higher neonatal ET-1 levels were associated with low Apgar score in newborns of mothers with abnormal umbilical Doppler studies, suggesting that ET-1 could be a marker of perinatal asphyxia.

Keywords: Endothelin-1, umbilical Doppler waveform, IDDM pregnant women.

Endothelin-1 (ET-1) seems to act as a local paracrine signal rather than a circulating hormone. Its effects are mediated by specific, membrane-bound receptors, which are detectable in high concentrations in the fetoplacental tissue. ET-1 causes an initial transient fall in blood pressure, followed by a strong, long-lasting increase in peripheral resistance and blood pressure[1]. This complex opposing vascular effects mediated through vascular smooth muscle and a number of receptor sub-types for endothelines namely ET-A, ET-B1 and ET-B2. ET-A and ETB2 are known to be selective for ET-1 and may be responsible for the direct vasoconstrictor activity. The ET-B1 receptors are believed to mediate vasodilator activity through release of endothelium derived mediators (PGI2, PGE2 and EDRF)[2].

The low placental vascular resistance is essential for efficient placental function; there by enabling it to meet the growing needs of the fetus right up until the end of pregnancy. Activation of vascular smooth muscle through generation of endogenous endothelin-1 appears to contribute to maintenance of blood pressure; basal vascular tone and regional blood flow[3].

Reduced output of nitric acid and prostacyclin with increased production of endothelin-1 and thromboxane A_2 are associated with placental insufficiency and fetal growth retardation .These changes promote platelet aggregation and intravascular coagulation, vasoconstriction, increased vascular sensitivity to vasoconstrictor stimuli, retarding blood flow and feto-placental growth. It was suggested that uteroplacental vascular bed, might be one of the sources for increased ET-1 production in women with pre-eclampsia[4,5].

Doppler velocimetry can supply important information about the uterine and umbilical circulation. Vascular impedance can be detected using umbilical artery Doppler flow-velocity waveform analysis. High S/D ratio more than 3, absent and reversed end diastolic flow are indices of vascular impedance[6]. Emily (2015)[7] showed that ET-1, and nor adrenalin related mechanisms could be involved in the abnormal umbilical artery velocity waveforms associated with pre-eclampsia.

Altered vascular responses to endogenous peptides such as endothelin-1 may be in part a cause of vascular dysfunction associated with diabetes. The decreased maternal peripheral resistance in pregnancy may be also a result of these alterations. Increased insulin resistance can activate the sympathetic nervous system and lead to an increased in expression of receptors for endothelin, both of which events lead to increased vasoconstriction[8,9].

Endothelin-1 is involved in the circulatory adaptation and in the transition from fetal to extra uterine life[10]. Elevated plasma levels of ET-1 during the neonatal period have been reported in different neonatal diseases[11].

Our objective is to study the maternal and neonatal plasma concentration of endothelin- 1 in relation to abnormal umbilical artery Doppler flow-velocity waveforms in pregnancies complicated with IDDM and its relation to the neonatal outcome.

Patients and methods

The study was carried out at Antenatal Care Clinic, National Research Center and El-Galaa Teaching Hospital. After taking consent, 60 singleton term normotensive IDDM pregnant women were chosen to join this study according to Doppler waveform study results. All women were subjected to history taking, medical and obstetrical examinations and routine investigations were done. In addition, they were subjected to ultrasonic assessment of fetal biometry, biophysical profile, placental site and grading together with Doppler studies of the umbilical artery. All women were delivered by cesarean section. Women recruited for this study, were divided into two groups: Group 1 (study group): 30 IDDM pregnant women with abnormal umbilical artery waveforms. Flow in the umbilical artery waveforms are considered abnormal if S/D ratio >3, absent or reversed end diastolic flow. Group II (control group): 30 IDDM pregnant women with normal umbilical artery flow velocity waveform studies. All pregnant women were submitted to laboratory investigations including: Fasting and 2 hours postprandial blood glucose by enzymatic methods using kits from Biomerieux (69280 Marcy-L, Etoile, France). Determination of glycosylated hemoglobin by ion exchange chromatography using kits from Stanbio Lab, Inc. (2930 East Houston st., San Antonio, Texas). Plasma concentration of ET-1 were measured in maternal venous blood before induction of anaesthia and neonatal umbilical cord blood in the two groups using a Sandwich Enzymatic Immunoassay technique from American Diagnostic Inc. (P.O. Box 1165, Greenwich, CT 06836-1165) . The kit uses monoclonal antibodies directed against endothelin-1. The detection limit was 0.1 Pg/mL. Neonatal assessment using the 5 minutes Apgar score and birth weight were estimated.

Statistical Methods

The collected data were coded, presented as mean ± SD. Statistical analysis using students t-test and Pearson Correlation Coefficient. Results were considered statistically significant if P value < 0.05.

RESULTS

Characteristics of mothers in both groups were represented in Table 1. No differences were found related to maternal age, gestational age or diabetic control using glycosylated hemoglobin.

Table 2 shows that the ET-1 levels in group I was ranging from 12.8 to 16.4 Pg/mL with a mean ± SD 14.24 ± 1.18 and in group II ranging from 12.0 to 16.21 Pg/mL with a mean ± SD 14.02 ± 1.3. This shows that there is no significant statistical difference of ET-1 levels between diabetics with abnormal flow velocity Doppler waveform and those with normal Doppler waveform.

Table 3 shows that the ET-1 levels in newborns of group I was ranging from 25 to 31.9 Pg/mL with a mean ± SD 28.7 ± 1.77 and in newborns of group II ranging from 25.6 to 29.4 Pg/mL with a mean ± SD 27.44 ± 1.01. This shows a highly significant statistical difference of ET-1 levels between newborns of diabetics with abnormal flow velocity Doppler waveform and newborns of diabetics with normal Doppler waveform ($P<0.001$).

Neonatal 5 minutes Apgar score and birth weight were compared in Table 4. The 5 minutes Apgar score of newborns of group I was ranging from 5 to 9 with a mean ± SD 7.03 ± 1.09, which was lower than that of newborns of group II that was ranging from 7 to 9 with a mean ± SD 8.03 ± 0.76. This shows that there is a highly significant statistical difference of Apgar score between newborns of diabetics with abnormal flow velocity Doppler waveform and newborns of diabetics with normal Doppler waveform.

The birth weight of newborns of group I was ranging from 3100 to 4100 gm with a mean ± SD 3400 ± 289 which was lower in comparison to the birth weight of group II which ranged from 3350 to 4200 gm with a mean ± SD 3830 ± 211. So a highly significant statistical difference of birth weights between newborns of diabetics with abnormal flow velocity Doppler waveform and newborns of diabetics with normal Doppler waveform.

Table 5 shows the ET-1 levels in neonates of group I in relation to the Apgar score. The ET-1 levels in newborns delivered with Apgar score <7 was ranging from 27.5 to 31.9 Pg/mL with a mean ± SD 30.47 ± 1.63 which was higher than ET-1 levels of newborns delivered with Apgar score >7 which ranged from 25 to 31 Pg/mL with a mean ± SD 28.16 ± 1.45. So in IDDM with abnormal flow velocity Doppler waveform, it was found that a highly significant statistical difference of ET-1 level between newborns with Apgar score <7 and newborns with Apgar score >7.

Table 1. Characteristics of Mothers of Both Groups

	Group I (N =30)	Group II (N=30)	P-value
Maternal age (years)	23.1 ± 2.51	21.6 ± 2.85	0.104 NS
Gestational age (weeks)	37.55 ±0.94	37.9 ± 1.7	0.26 NS
Glycosylated HB (%)	7.14 ± 0.81	6.83 ± 0.94	0.04 NS

Table 2. Comparison of Maternal Endothelin-1 levels In Both groups.

	Group I (N =30)	Group II (N =30)	P-value
ET-1 Mean (Pg/mL)	14.24	14.02	0.37
SD	1.18	1.30	NS

Table 3. Comparison of Neonatal Endothelin-1 levels In Both groups

	Group I (N =30)	Group II (N =30)	P-value
ET-1 Mean (Pg/mL)	28.7	27.44	0.001
SD	1.77	1.01	HS

Table 4. Comparison of Neonatal Outcome in Both Groups

		Group I (N=30)	Group II (N=30)	P-value\
Apgar Score	Mean	7.03	8.03	
	SD	1.09	0.76	0.001
Birth weight	Mean (gm)	3460	3830	HS
	SD	289.29	211.96	

Table 5. Comparison of ET-1 level in Relation to Apgar score in Neonates of group I

	Newborns with Apgar score < 7 (Group I) N=7	Newborns with Apgar score > 7 (Group I) N=23	P-value
ET-1 Mean (Pg/mL)	30.47	28.16	0.001
SD	1.63	1.45	HS

In-group I there were significant negative correlations between neonatal ET-1 level and both 5 min Apgar score (r= -0.39, P= 0.03) and birth weight (r = -0.40, P = 0.02). Meanwhile, no correlation could be detected between maternal ET-1 level and either neonatal ET-1, Apgar score, or birth weight in group I and in group II.

DISCUSSION

It is well established that women with diabetes had significantly higher levels of plasma ET-1 through pregnancy when compared to normal nondiabetic pregnant women[12]. Pregnant women with IDDM are known to be at a higher risk of developing uteroplacental vascular changes associated with placental insufficiency [(13)]. Diabetes is associated with vascular dysfunction, which may be due to altered vascular responses to endogenous peptides such as ET-1.

This study showed that there was no significant statistical difference of ET-1 levels between diabetics with abnormal flow-velocity Doppler waveform and those with normal Doppler waveform. This shows that ET-1 is not responsible for the placental vascular changes associated with diabetes. This may be explained by the abnormal vascular reactivity associated with diabetes in the form of reduced sensitivity to ET-1. This was also found by Elzbiet *et al*.[14] whereas sensitivity of myometrial blood vessel to endothelin- 1 was reduced in the diabetic compared with the nondiabetic pregnant women. Francesco *et al*.[15] found that there was no significant difference in plasma ET-1 levels between pregnant women with diabetes who had pre-existing diabetic retinopathy reflecting pre-existing endothelial damage and those without. They concluded that no association could be demonstrated between diabetic retinopathy and serum ET-1 levels. Also Furuya *et al*.,[1] found that IDDM pregnant women had markedly elevated ET-1 levels. However diabetic women with and without preeclampsia did not differ with respect to ET-1 concentration.

In our study ET-1, levels in the umbilical cord were higher than in the maternal plasma. Gospodins *et al*. [(16)] also found this. It is interesting to speculate that this increase in ET-1 concentration is fetal in origin.

In addition, we elicited a highly significant statistical difference of ET-1 levels and Apgar score between newborns of diabetics with abnormal flow velocity Doppler waveform and newborns of diabetics with normal Doppler waveform. Also we found a higher ET-1 levels in newborns with Apgar score < 7 compared to those > 7. A pathogenic role of perinatal asphyxia as a potent trigger for ET-1 synthesis and secretion could be confirmed by elevated ET-1 plasma levels in these neonates, which was also found by Laforgia *et al*. [(10)] and Neslihan *et al*.[17].

Although there was no difference in the diabetic control proved by glycosylated hemoglobin, a highly significant statistical

difference of birth weight between newborns of diabetics with abnormal flow-velocity Doppler waveform and newborns of diabetics with normal Doppler waveform was found. This could be attributed to decreased uteroplacental blood flow in neonates of mothers with placental insufficiency[18, 19, 20].

CONCLUSION

Although IDDM pregnant women have markedly elevated ET-1 levels, it is not responsible for the vascular changes associated with abnormal umbilical Doppler studies. Higher neonatal ET-1 levels associated with low Apgar score were found in newborns of mothers with abnormal umbilical Doppler studies, suggesting that ET-1 could be a marker of perinatal asphyxia.

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