Correlating the Blood Flow Class of Abnormal Umbilical Artery Doppler to Maternal Serum S100 b Protein and Endotheline-1 in Intrauterine Growth Retarded Fetuses

DOAA SALAH EL-DIN, M.D.*; HALA A.E. WAHAB, M.D.*; EMAN ZEIN, M.D.*; ABD EL-GHANY MOHAMED, M.D.* and MARWA M. SHETA, M.D.**
The Departments of Obstetric & Gynecology* and Clinical & Chemical Pathology**, Kasr El-Aini Hospital, Cairo University

Abstract

Background: Intra Uterine Growth Retardation (IUGR) is defined as birth weight below the 10th percentile for gestational age. Doppler ultrasonography is one of the principal surveillance tools in pregnancies complicated by IUGR. Abnormal Doppler findings are usually investigated as a group without taking into consideration the degree of abnormality, thus using a scoring system for the umbilical artery wave forms can differentiate the degree of severity. The aim of the study is to correlate the umbilical artery Doppler blood flow class to maternal serum endothelin-1 and S100 b protein in pregnancies complicated with IUGR.

Materials and Methods: 75 Patients pregnant between 30-37 weeks were divided into 2 groups: Study group which included 30 patients with pregnancy complicated by IUGR, and the control group which included 45 women, with uneventful pregnancies. Blood sample collection for measuring serum endothelin-1 and S100 b protein was done. Umbilical artery Doppler recordings were performed. PI was calculated and the blood flow waveform was classified as blood flow class (BFC) 0-3. BFC 0 stands for normal umbilical artery blood flow velocity waveforms. Abnormal umbilical artery Doppler included BFC 1-3, where BFC 3 is absent or reversed end diastolic blood flow (ARED).

Results: Ten patients were excluded from the study. Maternal plasma ET-1 and S 100 b protein concentrations were high in IUGR group compared to control group (p value <0.001). Of the IUGR group 21 patients showed abnormal Doppler findings. Patients with ARED (BFC 3) had mean maternal serum ET-1 level of 19±1.1pmol/L and mean S100 b protein of 0.657±0.114ug/L. Both values showed significant difference from control (p value: <0.001 and 0.009 respectively). While mean serum ET-1 level and S100 b protein in BFC 0, 1 and 2 showed no statistical difference between the 2 groups. Six cases in the IUGR group were admitted to neonatal intensive care unit, all had S100 b protein above 0.72ug/L. Four cases had BFC 3, 1 case BFC 1 and 1 case BFC 2. A positive correlation was found between endothelin-1 level and BFC 3, r=0.71, p value: 0.016. Also S100 b protein showed positive correlation to BFC 3, r=0.536, p value=0.068.

Conclusion: Umbilical artery Doppler BFC correlates well to ET-1 level and S 100 b protein and both are increased in IUGR foetuses. IUGR foetuses should not be studied as one group to avoid misleading results. Umbilical artery Doppler scoring system together with endothelin-1 level correlates to severity in IUGR while S 100 b protein has a good predictive value concerning NICU admission.

Key Words: Intra uterine growth retardation – Umbilical artery Doppler – Endotheline-1 and S100 b protein.

Introduction

INTRA Uterine Growth Retardation (IUGR) is defined as birth weight below the 10th percentile for gestational age [1]. Studies into the pathological process of IUGR have shown that abnormal placental function is a common mechanism [2]. Evidence now suggests that the placental dysfunction is often gradual and chronic. The decreased placental function can often occur much earlier than any demonstrable IUGR. At least two factors are known to contribute to the abnormal placental function. These include abnormal trophoblastic invasion and abnormal expression of vasoactive mediators [3]. IUGR is a major determinant of perinatal morbidity and mortality. Much of routine prenatal care involves detecting women at increased risk of this adverse event and targeting intensive monitoring and interventions. Standard reviews of fetal physiology suggested that variation in human fetal growth was largely a phenomenon of the second half of pregnancy and it is during this phase of pregnancy when women receive the bulk of prenatal care [4]. Despite relevant progress in obstetric clinical care IUGR is associated with increased perinatal mortality and accounts for 40% of neurologically damaged children [5]. Doppler ultrasonography is one of the principal surveillance tools in pregnancies complicated by placental
vascular insufficiency and IUGR [6]. This antenatal testing modality aims to detect fetal compromise by evaluating fetal manifestations of altered oxygenation and metabolic status [7]. The risk for adverse perinatal outcome in growth retarded fetuses were highest when Doppler examinations were worst, the degree of increasing vascular impedance was significantly related to increasing frequency of adverse outcome [8]. A normal Doppler examination of any small for gestational age (SGA) fetus is currently taken to suggest a small normal baby who is not at increased risk for growth retardation complications [9]. Although there is evidence that SGA babies with normal umbilical artery Doppler do not have same outcome as the general population [10], a normal Doppler has come to be used as a reassuring sign and a method to distinguish between constitutional and pathological smallness [11]. Abnormal Doppler findings are usually investigated as a group without taking into consideration the degree of abnormality. Pooling the finding may hide differences of clinical significance between more and less severely growth retarded fetuses. Thus using a scoring system for the umbilical artery wave forms can differentiate the degree of severity. Numerous serum markers that are measured in the maternal circulation have been evaluated in the perdition of IUGR, its severity and perinatal complications. Activin A, Inhibin A, follistatin, adrenomedullin, adiponectin, Leptin, endothelin-1 and S 100 b protein are among those factors [12-16].

Endothelin-1 (ET-1) is a vasoactive mediator produced primarily by endothelial cells; it is the most potent vasoconstrictor known, with its receptors expressed in the placenta. ET-1 secreted by vascular endothelial cells acts principally on the underlying smooth muscle and thereby affects local flow [17]. Thus, ET-1 synthesized in the placenta could regulate fetoplacental hemodynamics. Hypoxia is one of the main stimuli for ET-1 release. Although an increased contractile response of placental vessels to ET-1 has been claimed as the mechanism for development of IUGR [18], the exact pathway causing placental vasoconstriction is unknown. Also fetal pre-exposure to adverse intrauterine conditions, such as decreased oxygen and substrate supplies that occur in IUGR play a causal role in perinatal mortality and central nervous system injury especially intraventricular hemorrhage [5]. S 100 b protein is an acidic calcium-binding protein found in the glial cells, astrocytes, Schwann cells, and neurons, it is thought to be involved in the regulation of several cellular functions (cell-cell Communications, cell growth, cell structure, energy metabolism, contractions and intracellular signal transduction) [19]. The findings of increased S 100 b protein concentration in biological fluids (e.g. cerebrospinal fluid, blood and amniotic fluid) of adults, infants and fetuses after cell injury in the nervous system have supported the use of S 100 b as a biochemical marker of brain damage [20-22]. Several studies showed that S 100 b concentration was significantly higher in pregnancies complicated by IUGR [16-23].

The aim of the study is to correlate the umbilical artery Doppler blood flow class to maternal serum endotheline-1 and S 100 b protein in pregnancies complicated with IUGR.

Material and Methods

The study was conducted at Kasr El-Aini Hospital in the duration from November 2008 to June 2009. The study group (1) included 30 patients with pregnancy complicated by IUGR among those attending obstetric outpatient clinics in Kasr El-Aini Hospital. IUGR was defined by the presence of ultrasonographic signs (bi-parietal diameter below the 10th percentile and abdominal circumference below the 5th percentile and fetal weight below the 10th percentile for gestational age) in all cases [24].

The control group (2) included 45 women, who had uneventful pregnancies with ultrasound estimated fetal weight between the 10th and 90th percentile according to gestational age. All cases in the control group had normal velocity waveforms in the umbilical artery. Patients were followed up every 2 weeks until the 36th week and weekly thereafter. Informed consent was obtained from all women before inclusion in the study, and approval was obtained from local committee.

Inclusion criteria are:
- Single tone pregnancy,
- No Maternal gestational diabetes or hypertension,
- No Maternal central nervous system (CNS) illness,
- Gestational age between 30 - 37 weeks as determined by the last menstrual period and confirmed by a first trimester ultrasound scanning and
- No detected fetal congenital anomalies during ultrasound scanning.

Babies born prior to 37 weeks were excluded from the study. The study group was not involved in any decision concerning timing and mode of delivery of all cases. At delivery patients were examined for fetal weight, Apgar score at 1 minute and at 5 minutes as well as neonatal intensive care unit (NICU) admission.
Sample collection:

Samples of maternal venous blood (3ml) were collected into EDTA tube chilled into ice and immediately cold centrifuged 4°C at 1500g. Clear plasma was separated and kept at 20°C till analysis of the plasma ET-1 by radioimmunoassay kit supplied by Eurodagnostica (Netherlands) and serum S 100 b by enzyme linked immunosorbent assay (ELISA) provided by Cna Ag Diagnostics AB Gothenburg, Sweden as described previously by Michetti F and Gazzolo D [25].

Ultrasound studies:

Fetal biometry (abdominal and head circumference, biparietal diameter and femur length) as well as the flow velocity waveforms (FVW) in the umbilical artery in all pregnancies (both IUGR and the control groups) were measured. This was conducted by an accuvix XQ, (Medison, Korea) ultrasound machine with 3.5MHZ curvilinear array with available color and pulsed Doppler options. Umbilical artery Doppler recordings were performed in the absence of fetal breathing or movement. PI was calculated from three even subsequence blood flow velocity waveforms according to Gosling, et al. [26], and the blood flow waveform was classified as blood flow class (BFC) 0-3 defined as BFC 0: Normal umbilical artery blood flow velocity waveforms (PI ≤ 2 SD above the mean); BFC 1: PI between 2 and 3 SD; BFC 2: PI > 3 SD and forward diastolic blood flow and BFC 3: Absent or reversed end diastolic blood flow (ARED). Abnormal umbilical artery Doppler was defined as BFC of 1-3 [27].

Statistical methods:

Data were statistically described in terms of mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. A probability value (p-value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Ten patients were excluded from the study, three patients delivered before 36 weeks (2 from control group and 1 from IUGR group), 7 patients could not be assessed during delivery (patients delivered outside our hospital, 4 from the control group and 3 from IUGR group. Thus complete data were obtained from 26 patients in group 1 (IUGR group) and 39 patients in group 2 (control group).

Demographic and clinical characteristics of the study groups are shown in Table (1). As expected mean birth weight at delivery was lower in the IUGR group than in control group. Also the mean gestational age was lower in the IUGR group due to the need for earlier intervention either due to worsening of Doppler finding or for maternal reasons.

Maternal plasma ET-1 and S 100 b protein concentrations were high in IUGR group compared to control group, Table (2). The Doppler finding and BFC of the umbilical artery in the 2 groups is shown in Table (3).

Table (1): Demographic data.

<table>
<thead>
<tr>
<th></th>
<th>IUGR group (n=26)</th>
<th>Control group (n=39)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)*</td>
<td>27±2</td>
<td>28±3</td>
<td>0.141</td>
</tr>
<tr>
<td>BMI (Kg/m²)*</td>
<td>27.8±4</td>
<td>29.4±4.5</td>
<td>0.147</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)*</td>
<td>37.2±3.2</td>
<td>39.2±1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Birth Weight (gram)*</td>
<td>1840±150</td>
<td>3120±350</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apgar score at 1min.</td>
<td>6.4±2.1</td>
<td>8.5±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apgar Score at 5min.</td>
<td>9.4±0.8</td>
<td>9.7±0.5</td>
<td>0.067</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SD. BMI: Body mass Index.

Table (2): Maternal serum biochemical markers.

<table>
<thead>
<tr>
<th></th>
<th>IUGR Group (n=26)</th>
<th>Control Group (n=39)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum ET-1 (pmol/L)*</td>
<td>16.6±4.2</td>
<td>10.9±2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal serum S100b protein (ug/L)*</td>
<td>0.564±0.211</td>
<td>0.147±0.55</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Mean ± SD. ET-1: Endothelien level.

Table (3): Umbilical artery Doppler findings in the two groups.

<table>
<thead>
<tr>
<th></th>
<th>IUGR group (n=26)</th>
<th>Control group (n=39)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Doppler finding</td>
<td>BFC 0 (19.2%)</td>
<td>39 (100%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal Doppler findings</td>
<td>BFC 1 (23.1%)</td>
<td>0 (0.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BFC 2 (23.1%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BFC 3 (34.6%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Results are number (%). BFC : Blood Flow Class. IUGR: Intrauterine growth retardation.
Patients with ARED (BFC 3) had mean maternal serum ET-1 level of 19±1.1 pmol/L and mean S 100 b protein of 0.657±0.11 ug/L. Both values showed significant difference from control (p value: <0.001 and 0.009 respectively). While mean serum ET-1 level and S 100 b protein in BFC 0, 1 and 2 showed no statistical difference between the 2 groups (except BFC 2 and serum ET-1), Table (4), (Figs. 1, 2).

Six cases in the IUGR group were admitted to neonatal intensive care unit, all had S 100 b protein above 0.72 ug/L, 4 cases had BFC 3, 1 case BFC 1 and 1 case BFC 2, Table (5).

A positive correlation was found between endotheline-1 level and BFC 3 r=0.71, p value: 0.016. Also S 100 b protein showed positive correlation to BFC3, r=0.536, p value=0.068. Table (6), shows the correlation between BFC 3 and ET-1 level as well as S 100 b protein. No correlation was found between the other blood flow classes and the biochemical markers (p value >0.05).

The predictive values of Doppler findings and S 100 b protein in predicting NICU admission showed that S100 b protein above 0.72 ug/L had 100% sensitivity and 90% specificity. BFC 3 had 66.7% sensitivity and 75% specificity Table (7).

Table (4): Relation of the biochemical markers to Doppler finding.

<table>
<thead>
<tr>
<th>Doppler finding</th>
<th>IUGR group (n=26)</th>
<th>Control group (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BFC 0 (n=5)</td>
<td>BFC 1 (n=6)</td>
</tr>
<tr>
<td>Maternal serum ET-1 (pmol/L)</td>
<td>12.9±2.6</td>
<td>13.1±2.4</td>
</tr>
<tr>
<td>p-value</td>
<td>0.113</td>
<td>0.058</td>
</tr>
<tr>
<td>Maternal serum S 100 b Protein (ug/L)</td>
<td>0.281±0.12</td>
<td>0.302±0.23</td>
</tr>
<tr>
<td>p-value</td>
<td>0.594</td>
<td>0.503</td>
</tr>
</tbody>
</table>

BFC: Blood Flow Class of umbilical artery.
IUGR: Intrauterine growth retardation.
p-values (all compared to control).

Table (5): NICU admission among IUGR group.

<table>
<thead>
<tr>
<th>Serum S100 b protein &gt;0.72 (ug/L)</th>
<th>NICU admission to NICU</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum S100 b protein</td>
<td>(n=6)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>&gt;0.72</td>
<td>6 (100%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>BFC 1</td>
<td>1 (16.7%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>BFC 2</td>
<td>1 (16.7%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>BFC 3</td>
<td>4 (66.7%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>ET-1 (Pmol/L)</td>
<td>17.2±2.4</td>
<td>16±2.6</td>
</tr>
</tbody>
</table>

IUGR: Intrauterine growth retardation.
NICU: Neonatal intensive care unit.
BFC: Blood Flow Class of umbilical artery.

Table (6): Correlation between BFC 3 and maternal serum markers.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum endotheline-1</td>
<td>0.71</td>
<td>0.016</td>
</tr>
<tr>
<td>Maternal serum S 100 b protein</td>
<td>0.536</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Table (7): Predictive values of Doppler findings and S100 b protein in predicting NICU admission.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum S 100 b protein</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>BFC 1</td>
<td>16.7%</td>
<td>75%</td>
</tr>
<tr>
<td>BFC 2</td>
<td>16.7%</td>
<td>75%</td>
</tr>
<tr>
<td>BFC 3</td>
<td>66.7%</td>
<td>75%</td>
</tr>
</tbody>
</table>

BFC: Blood Flow Class of umbilical artery.
Discussion

IUGR results from persistent suppression of genetic growth potential that occurs in response to reduction in substrate supply. It is commonly accepted that IUGR is associated with an impairment of uteroplacental blood flow that may be reduced up to 50% as a result of impaired trophoblast invasion of spiral arteries that are not transformed to low-resistance vessels [28].

Doppler, especially umbilical artery Doppler flow is commonly used to determine the risk of adverse perinatal outcome for IUGR fetuses. Antenatal identification of these fetuses improves the outcome [29]. A normal antenatal umbilical artery Doppler cannot be taken as an indicator of low risk in pregnancies complicated by IUGR [30]. This brought into consideration the need for umbilical artery Doppler flow scanning (to differentiate the degree of risk and severity) as well as correlating these findings to other biochemical markers known to be increased in IUGR fetuses. ET-1 is a 21-amino acid polypeptide, potent vasoconstrictor that is produced and released primarily from the endothelial cells. It is also produced by glandular epithelium including the amnion cells. It has been demonstrated that the contractile response to ET-1 in placental vessels was increased in pregnancies complicated with IUGR compared to uneventful cases and, therefore, hypothesized that ET-1 might be involved in the pathophysiology of IUGR [31]. S 100 b protein is an acidic calcium binding protein previously demonstrated as a reliable indicator of a brain lesion. S 100 b protein concentrations are increased in several biological fluids in the presence of brain injury and pregnancies at high risk for chronic fetal hypoxia as IUGR fetuses [32].

The control group included women with uneventful pregnancies with absent maternal diabetes, hypertension or CNS illness also ultrasound findings of appropriate for gestational age fetuses and normal Doppler findings. This avoids any misleading results from maternal diseases or undiagnosed small for gestational age fetuses. The highest maternal plasma ET-1 concentration was found in pregnancies complicated by ARED flow in the umbilical artery (p value <0.001. Pregnancies complicated by IUGR, but with BFC 0 and 1 showed no elevation in ET-1 compared to control (p value 0.113 and 0.058 respectively). To our knowledge, no other study compared the maternal serum ET-1 level in IUGR fetuses to umbilical artery Doppler scoring system. Investigating the abnormal umbilical artery Doppler findings as a group without taking into consideration the relation between the severity of IUGR and the observed change in the Doppler findings results in pooling the findings together which may hide differences of clinical significance between more and less severely growth retarded fetuses needing more care. This also explains some contradictory results seen in different studies concerning the value of umbilical artery Doppler in IUGR fetuses.

Some studies showed little value of umbilical artery Doppler in predicting IUGR fetuses with increased risk of adverse perinatal outcome. The study done by Schiff, et al., 1994 showed higher fetal endothelin-1 level in IUGR regardless of umbilical artery Doppler measures [33]. Same finding was shown by Erdem et al., 2003 who examined the maternal and fetal ET-1 in pregnancies complicated with IUGR and correlated these data with umbilical artery Doppler flow velocity waveforms. They found higher mean maternal and fetal ET-1 levels in pregnancies complicated with IUGR than in controls but found that maternal and fetal ET-1 concentration were not related to umbilical artery Doppler flow PI, RI and S/D ratio [34]. Figueras, et al., 2008, in their study comparing the relationship between smallness at birth and the predictive value of umbilical artery Doppler, stated that fetuses that are small for gestational age are at increased risk of adverse outcome regardless of the results of umbilical artery Doppler [30].

On the other hand, many studies who investigated the role of ET-1 in the pathogenesis of IUGR have correlated the umbilical Doppler flow waveforms with the maternal and fetal serum levels. The results of the present study are in agreement with those studies [35-37].

In the study of Hartikainen et al., 1991 increased ET-1 levels have been detected in umbilical arteries from pregnancies with umbilical artery vasospasm as IUGR and pre-eclampsia and with absent end diastolic flow velocity using umbilical artery Doppler velocimetry [35]. Karsdrop, et al., 1998 studied the plasma concentrations of endothelin, Lipidhydroperoxides, glutathione peroxidase and fibronectin in relation to abnormal umbilical artery velocimetry in different patients. The study concluded that abnormal Doppler velocimetry; especially ARED flow is associated with elevated maternal and fetal plasma levels of ET-1 [36]. Ghosh and Gudmundsson in their study stated that Doppler examination of the uterine and/or umbilical arteries seem to be comparable as predictors of outcome in pregnancies complicated by IUGR. There was a statistically significant correlation between abnormal Doppler of the uterine and/or umbilical arteries and adverse outcome of pregnancy [38].
In our study decreased or absent end diastolic flow velocities reflecting increased vascular resistance in the feto-placental unit is associated with the highest level of maternal serum ET-1 and S100b protein. (p value compared to control was: <0.001 and 0.009 respectively). High maternal serum S100b protein was seen in IUGR group with p value <0.001 compared to control. This is in accordance with the results of other studies [13,16]. The cut off point for defining high maternal serum S100b protein was chosen by Roc curve analysis to be 0.72 µg/L. Considering this cut off value in our study diagnosed 100% of NICU admission (6 cases). Further assessment is needed to investigate this finding on a larger scale of patients. No difference found between ET-1 level in IUGR group admitted or not to NICU (p value: 0.324). BFC 3 diagnosed about 66% of cases in need to NICU admission.

It was noted that the 5 cases in IUGR group with BFC 0 had maternal serum ET-1 and S100b protein with no significant difference to control (p value: 0.113 and 0.594 respectively) and no case needed NICU admission. Finally, umbilical artery Doppler BFC correlates well to ET-1 level and S100b protein and both are increased in IUGR fetuses. IUGR fetuses should not be studied as one group to avoid misleading results. Umbilical artery Doppler scoring system together with endothelin level correlates to severity in IUGR while S100b protein has a good predictive value concerning NICU admission.

References


