Morphological Placental Portraits in Congenital Diaphragmatic Defects: A Pathological Study Approach

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Abstract

Introduction: The pathogenesis of Congenital Diaphragmatic Defects (CDD) is not clear. Placental factors have been implicated in the pathogenesis of congenital anomalies.

Objective: To assess the placental measurements on CDD and to compare with a group of fetuses without CDD.

Material and Methods: In this retrospective cross-study, 30 placentas of fetuses with CDD sent to Embryo fetal Pathology Laboratory, Centro de Genética Clínica (CGC), Porto, was evaluated. To compare the placental parameters in CDD group, 71 placentas from fetuses without CDD, matched by Gestational Age (GA) were selected.

Conclusion: Placental size and umbilical cord length and diameter have an impact on CDD.

Keywords: Congenital diaphragmatic defects; Diaphragmatic agenesis; Diaphragmatic eventration; Placenta size; Umbilical cord length; Umbilical cord diameter

Introduction

Congenital Diaphragmatic Defects (CDD) are rare anomalies, which includes a spectrum of defects such as Diaphragmatic Eventration (DE) and Diaphragmatic Hernia (DH). Besides the existence of a defect in the diaphragm, the abdominal contents protrude in to the thoracic cavity [1,2]. Development of the diaphragm starts during the 4th week of gestation and is completed by the 8th week. However, in the case of fetuses with diaphragmatic defects, the diaphragm doesn’t close completely during this period [3,4]. Congenital Diaphragmatic Hernia (CDH) is a developmental anomaly, with a mean incidence of 1:2.500 births [1,2]. It is considered as the major life-threatening cause of respiratory failure in newborns. Therefore, prenatal and postnatal clinical management remains a challenge [1-4]. About 5% of CDD cases represent Diaphragmatic Eventration (DE), a condition resulting in a reduced muscle thickness that allows tenting of the diaphragm in to the thoracic cavity. Usually, it is characterized by a diaphragmatic defect that allows intra thoracic herniation of abdominal organs and consequently lung hypoplasia, or lung alveoli and pulmonary vessels deviations. At birth, this leads to respiratory insufficiency and persistent pulmonary hypertension [1-5].

CDH more over is associated with diaphragmatic agenesis or large diaphragmatic muscle defect. About 85% of cases of CDH area left-sided posterolateral location through foramen of Bochdalek and less than 2% take place in other locations [2,6]. Clinically classified as either isolated, syndromic or associated with other anomalies [2]. Infact, the CDH prognosis depend on the presence of other associated fetal anomalies including central nervous system, gastrointestinal tract, skeletal system, genital urinary and heart defects [2,3,6,7].

The processes involved in CDH pathogenesis are still poorly understood [7]. Some studies suggest that these processes are determined by abnormal gene expression that recapitulates important event sin embryonic lung and pleuroperitoneal membrane development [7-9]. A few familiar cases described suggest a possible autosomal recessive transmission in this pathogenesis [7]. Aneuploidy, usually trisomy 21 or 18, occurs in 4% of the cases of CDH and many CDH
may be part of a syndrome, such as Beckwith-Wiedemann, Fryns syndrome, Ivemark or Goltz syndrome [6,7]. Other studies suggest that mesenchymal dysplasia, hypoxic embryonic and placental environments are involved also [8-10].

CDD can be accurately diagnosed during the second trimester routine ultrasound examination [1,6]. The optimal antenatal risk stratification of CDD is necessary for earlier prenatal counseling, personalized prognosis and appropriate perinatal and postnatal management [2,4,5,7].

CDH is a malformation which arises during the organogenesis period that affects to the development of pleuropertitoneal membrane and consequently thoracic abdominal wall. So, it is possible that umbilical cord maldevelopment can be a biologically plausible hypothesis involved in CDD [7,11-14].

Hence, the aim of this study is to investigate placental features in CDD and predict a potential target in early prenatal diagnosis.

**Materials and Methods**

We carried out a prospective pathological study in CGC Genetics embryofetal pathology laboratoy between January 01, 2011 and December 31, 2016 in order to select placental pathological reported related with a fetal diaphragmatic defects cases. In 2,903 fetal/perinatal autopsy reports, we identified 30 CDD cases (1.03%) which 22 cases correspond a CDH and 8 cases a DE. Gestational age ranged from the 11th and the 40th week. Seventy-one placentas (1.6%), from fetus without CDD, matched by GA were selected from a sample of 4,492 placentas cases in the same period. Two Edwards’s syndrome without CDD and one case of cardiac anomaly were included in group without CDD for comparison with the similar cases of CDD group.

Statistical analysis of the data was done using the statistical software IBM® SPSS® Statistics version 24.0. Given the nature of the variables involved, we opted for the use of statistical tools most appropriate to the measurement scales used. Therefore, in the descriptive study of the data-qualitative and quantitative variables (bar and pie charts, frequency tables, mean, median, standard deviations, minimum, maximum and box plot graphs) were used. In the analytical study of the data as relationship between two variables, the chi-square test (qualitative variables) was used. In the comparative study to evaluate differences between groups, one way ANOVA techniques was used.
when the variables presented normal behavioral their equivalent and Kruskal-Wallis when the normality assumption is not verified he Shapiro-Wilk test was used to evaluate the adjustment to normality. The decision of significant statistical evidence for probability values (p-value) was less than 0.05.

Pathological studies were conducted according to published recommendations [15,16]. Placentas were initially examined macroscopically, for photos acquisition and gross parameters evaluation. Placental disk measures were achieved in all groups: placenta weight (g), disk diameters and thickness and size (cm)-umbilical cord length and diameter (cm)-cord insertion type and number of umbilical vessels. Cord insertion type was classified as normal, where there was a central or eccentric insertion. Pathologic insertion was considered when it was marginal (<1 cm from the nearest margin) and velamentous (when drawstring route in the membranes) [16].

In CDH and DE groups several fetal defects were studied for each case individually: lung malformation, congenital heart disease, thoracic-abdominal wall defect, urogenital anomaly, endocrine anomaly and neural tube defect and in the same way with the 3 syndromic cases of the group of placentas without CDD. The number of defects was compared in the three groups, and the differences were evaluated by analysis of variance (ANOVA test).

Selected fragments for histological study based on the macroscopic data, clinical information and following pathological protocols, were sampled, prepared, sectioned at 5 μm and stained with Hematoxylin-Eosin (H & E) to allow the pathological diagnosis in placenta and fetal samples [16].

All the samples enrolled in the present study were unlinked and unidentified from their donors, and fulfilled the international ethics recommendations for Medical Research in Humans of the Declaration of Helsinki, the World Health Organization and the European Community, to be used in this study. The Ethical Review Committees of the involved institutions and Medicine School of Minho University approved the work and waived the need for written informed consent. Patients authorized the realization of pathological study in CGC Genetics, Porto, as well as all the procedures needed for pathologic diagnosis education and scientific research.

**Results**

Of the 101 cases evaluated, Table 1 summarizes the distribution cases in the groups according to the evaluated placental and cord parameters, type of delivery, syndromic or non-syndromic cases. Thirty cases presented CDD, 8 (7.9%) corresponding a DE and 22 (21.8%) a CDH of which 16 were on the left side. Fetal gender in 31 (34.4%) cases were female and in 40 (44.4%) cases were male and in 30 cases is unknown. In order to evaluate the association of CDD with fetal syndrome, a chi-square independence test was performed, with a statistically significant association ($\chi^2=35.090$, DF=2, p<0.001) in that the presence of syndrome is associated with CDH. This result is illustrated in the graph of Figure 1. For the comparison of the quantitative measures (placenta diameter, cord length, placenta weight and cord diameter) according to the group, the data normality was assured by the analytical test. In the placental weight parameter, the data normality was done through Kruskal Wallis. After completing the multiple comparison tests, and given the existence of a control group, Dunnett’s test revealed that: 1. There were significant differences in the mean placental diameter value when we compared the CDH group with the control (p=0.008) in that the mean-value of the CDH group was significantly lower; 2. There were significant differences in the mean-value of the placenta diameter when we compared the DE group with the control (p=0.008) in that the mean-value of the DE group was significantly lower; 3. There were significant differences in the mean-value of umbilical cord length when we compared the CDH group with the control (p=0.01) in that the mean-value of the CDH group is significantly lower; 4. There were significant differences in the mean-value of umbilical cord length when we compared the DE group with the control (p=0.001) in that the mean-value of the DE group is significantly lower; 5. There were significant differences in the mean-value of umbilical cord diameter when we compared the CDH group with the control (p=0.005) in that the mean-value of the CDH group is significantly lower; 6. There were significant differences in the mean-value of umbilical cord diameter when we compared the DE group with the control (p=0.012) in that the mean-value of the DE group is significantly lower.

To evaluate placental weight on the groups, the Kruskal-Wallis test was performed. There were significant differences in the mean-value of the placenta weight due to the group to which it belongs ($\chi^2=16.694$, DF=2, p<0.001). Multiple comparison tests revealed that 1. There were as significant differences in the mean-value of placentat weight when we compared the CDH group with the control (p=0.002) in the sense that the mean-value of the CDH group was significantly lower; 2. There were significant differences in mean placental weight when we compared the DE group with the control (p=0.025) in the sense that the mean-value of the DE group was significantly lower. All
these results are illustrated in the graph of (Figure 2).

It was detected significant association between CDD and umbilical vessels ($\chi^2$=24.736, p<0.001), other congenital defects ($\chi^2$=29.052, p<0.001). There were no significant associations between CDD and cord insertion, umbilical knots and membranes insertion type (p>0.05).

Figure 3 illustrate the statistically significant association between CDD and umbilical vessels. In that the existence of 2 vessels was associated with the CDH group. Also there was a statistically significant association between CDD and the existence of other defects, in that other defects was associated with the CDH group.

Discussion

In the present study oneway ANOVA verified significant differences in the mean-value of umbilical cord lengths and umbilical cord diameters in CDD and control groups, (F (2,92)=10.464, p<0.001) and (F (2,93) =7.710, p=0.001), respectively. Umbilical cord characteristics are well determined [15,16]. Also, umbilical cord anomalies are well described as well as the association with fetal anomalies or genetic defects [11,12,17,18].

Excessively thin umbilical cords are associated with fetal problems, as fetal growth restriction. A thick umbilical cord are associated with maternal diabetes and fetal macrosomia and fetal hydrops [17,18]. Nevertheless, to the best of our knowledge, no previous association has been described between decreased diameter of the umbilical cord and CDD. Our study documented-significant differences in the mean-value of umbilical cord diameter when we compared the CDH and DE groups with the control (p= 0.005) and (p=0.012) respectively. In fact, the mean-value of cord diameter in CHD and DE groups are significantly lower when compared to the control group.

There are many studies documenting associations between umbilical cord length and fetal congenital malformation, namely CDH [11-26]. In our study, the mean-value of umbilical cord length was significantly lower when we compared the CDH and DE groups with the control (p=0.006) and (p=0.001) respectively. UC length showed a greater difference in the DE group possibly related with abdominal and/or thoracic-abdominal wall defects as omphalocele, ectopia cordis, and limb body stalk complex seen in these groups [11-14]. Single Umbilical Artery (SUA) occurred in 40% of CDH group and 2.8% in control group. Prevalence of SUA is higher in congenital and structural fetal anomalies and chromosomal defects compared with 0.5% to 1% incidence in normal singleton. Higher prevalence of SUA in CDH group may be related to fetal pathology associated in this group. Pathologic cord insertion is associated with congenital anomalies when compared with normal pregnancies in which velamentous and marginal cord insertion in general occurs around 1% and 7% correspondingly [25,27]. Our results documented a higher number of pathologic cord insertion in DE and CDH groups with 14.3% and 5.6% respectively.

Normal placental function is critical to optimize fetal growth and development. Some authors documented a decrease in placental size with a hypoxic environment and placental hypoxic injury, namely fetal vessels lesions [16,28-32]. Placental size deviation has

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Table 1: Characterization of clinical and pathological evaluated parameters in congenital diaphragmatic defects and control groups, given in absolute evaluate (n) and percentage (%).

<table>
<thead>
<tr>
<th></th>
<th>CDD</th>
<th></th>
<th>DE</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Without</td>
<td>CDH</td>
<td>DE</td>
<td></td>
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<tr>
<td>Delivery type, N (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MT</td>
<td>9 (12.7)</td>
<td>16 (72.7)</td>
<td>7 (87.5)</td>
<td>32 (31.7)</td>
</tr>
<tr>
<td>FD</td>
<td>38 (53.5)</td>
<td>4 (18.2)</td>
<td>1 (12.5)</td>
<td>43 (42.6)</td>
</tr>
<tr>
<td>NND</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>NB</td>
<td>24 (33.8)</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>25 (24.8)</td>
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<tr>
<td>Syndrome, N (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>68 (95.8)</td>
<td>9 (40.9)</td>
<td>5 (62.5)</td>
<td>82 (81.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>3 (4.2)</td>
<td>13 (59.1)</td>
<td>3 (37.5)</td>
<td>19 (18.8)</td>
</tr>
<tr>
<td>Cord insertion, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>57 (80.3)</td>
<td>13 (72.2)</td>
<td>4 (57.1)</td>
<td>74 (77.1)</td>
</tr>
<tr>
<td>Marginal</td>
<td>14 (19.7)</td>
<td>4 (22.2)</td>
<td>2 (28.6)</td>
<td>20 (20.8)</td>
</tr>
<tr>
<td>Velamentous</td>
<td>0 (0)</td>
<td>1 (5.6)</td>
<td>1 (14.3)</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Umbilical vessels, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three</td>
<td>69 (97.2)</td>
<td>12 (60)</td>
<td>8 (100)</td>
<td>89 (89.9)</td>
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<tr>
<td>Two, 4+</td>
<td>2 (2.8)</td>
<td>8 (40)</td>
<td>0 (0)</td>
<td>10 (10.1)</td>
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<td>Umbilical knots, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>62 (87.3)</td>
<td>18 (100)</td>
<td>7 (100)</td>
<td>87 (90.6)</td>
</tr>
<tr>
<td>False Knots</td>
<td>9 (12.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>True Knots</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Membranes insertion, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>66 (93)</td>
<td>18 (85.7)</td>
<td>6 (85.7)</td>
<td>90 (90.9)</td>
</tr>
<tr>
<td>Circumvallate</td>
<td>5 (7)</td>
<td>3 (14.3)</td>
<td>1 (14.3)</td>
<td>9 (9.1)</td>
</tr>
<tr>
<td>GA (weeks), (SD)</td>
<td>27.1 (9.6)</td>
<td>20 (8.4)</td>
<td>15.9 (4.3)</td>
<td>24.7 (9.8)</td>
</tr>
<tr>
<td>Placenta diameter (cm), M (SD)</td>
<td>13.8 (4.4)</td>
<td>10.5 (4.2)</td>
<td>8.9 (3.2)</td>
<td>12.8 (4.6)</td>
</tr>
<tr>
<td>Cord length (cm), M (SD)</td>
<td>30.5 (14.6)</td>
<td>19.2 (11.2)</td>
<td>10.1 (5.8)</td>
<td>26.9 (14.9)</td>
</tr>
<tr>
<td>Cord diameter (cm), M (SD)</td>
<td>1.1 (0.5)</td>
<td>0.7 (0.3)</td>
<td>0.6 (0.3)</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

N: Count; %: Percentage; M: Mean; SD: Standard Deviation; CDD: Congenital Diaphragmatic Defects; CDH: Congenital Diaphragmatic Hernia; DE: Diaphragmatic Eventration; MT: Medical Termination of pregnancy; FD: Fetal Death; NND: Neo Natal Death; NB: Newborn; GA: Gestational Age dated of the 1st day of last menstrual period.
been related with morbidity and mortality in the fetus [16]. This may provide relevant information to the management of fetal and perinatal outcomes [16,32,33]. Our study documented a significantly lower mean of placental diameter value in CDH and DE groups when compared with the control group (p=0.008). Placenta weight is well established as well as its correlation with fetal abnormalities [16]. The mean-value of placental weight is significantly lower in CDH group and DE group when compared with the control (p=0.002) and (p=0.025).

**Conclusion**

The results herein reported suggest that placental disk and umbilical cord measurements could be a risk predictor of fetal anomalies that must rule out the presence of congenital diaphragmatic defects.

The pathological measurements, could improve the ultrasound parameters accuracy after an appropriated protocol and serial statistical approach.

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CGC genetics, Porto, Portugal.

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