Effects of uterine cervix constriction on Wistar rats

Cassio Eduardo Raposo-Amaral, Ana Beatriz Almeida, Cesar Augusto Raposo-Amaral, Luiz Carlos Vulcano, Maria Rita Passos-Bueno, Nivaldo Alonso

ABSTRACT

Purpose: To verify if uterine cerclage can induce craniosynostosis or any cranial deformity in new born Wistar rats. Methods: One pregnant female Wistar rat underwent laparotomy on day 18 of gestation and the uterus cervix was closed with a 3-0 nylon suture to avoid delivery, that occurs normally on the 21 day. The suture was released after 48 hours beyond the normal gestation period. The female rat delivered 11 pups. Six surviving rats from the delivery (group A - constrained group). Two rats were born from another mother and in the same age were used as control group (group B - 2 nonconstrained controls) were allowed to grow. They were sacrificed 1.2 years after their birth all the eight animals. Linear measurement, routine histology and computed tomography of the skull were performed at the time of their death to evaluate the cranial asymmetries by measurements of the anatomical landmarks of the craniofacial skeleton of the rats on the two groups and compared then. Results: We did not observe statistically significant differences in any of the compared measurements (p>0.05) obtained through the morphologic and radiologic methods. Histologic examinations did not reveal any sign of premature fusion or suture imbrications. Critical decrease in longitudinal body size was noticed as the limbs too in all the animals of group A. Conclusion: Constriction of uterine cervix leads to fetus suffering, even death for a few animals, associated to small body size, but not to craniosynostosis.

Key words: Craniosynostoses. Cerclage, Cervical. Surgery, Plastic. Rats.

INTRODUCTION

Several theories explain the premature fusion of the cranial sutures in non syndromic craniosynostosis. Potential causes that may trigger the premature fusion of the cranial sutures are suggested and may vary from metabolic derangement to maternal hyperthyroidism, hypercalcemia, or idiopathic deficit in brain growth. Martinez-Lage et al.2 have linked neural tube defect to the premature fusion of the cranial sutures. They advocated that the decrease in intra cranial pressure and in brain pulsation may...
lead to suture imbrication, causing synostosis. However, mechanical forces that somehow constrict the fetal head during the third trimester of gestation are considered to be a main cause of non syndromic forms of craniosynostosis. Clinical studies have emphasized the fetal head constraint as an important etiology in craniosynostosis development.

Experimental studies have shown the effects of the replication of mechanical forces on producing premature fusion of the cranial sutures, by postponing the normal gestation period beyond 24 hours. Nevertheless, fetal head constraint is not widely accepted as a main etiology for cranial suture fusion in non syndromic craniosynostosis. Bradley et al. have tested the mechanical restrictive uterine forces to reproduce suture fusion in a lamb model. They have observed craniofacial skeleton deformation with patency of cranial sutures and not craniosynostosis.

The purpose of this study was to verify if uterine cerclage can induce craniosynostosis or any cranial deformity in Wistar rats.

Methods

Pregnant female Wistar rats underwent laparotomy on day 18 of gestation and the uterus cervix was closed with a 3-0 nylon suture under anesthesia, using an intramuscular injection (0.3 mL/100 g of body weight) with a combination of ketamine hydrochloride (5%) and xylazine (2%) (Figure 1).

The suture was released after 48 hours beyond the normal 21 days gestation period. Six hours after the suture was released, the female rats delivered 11 pups. Six of 11 pups survived the severe maternal stress. Five dead pups were removed from their mothers and compared to nonconstrained controls, which had been delivered normally on day 21 of gestation. The craniofacial skeleton of the five dead pups received gross inspection and the skull specimens were sent to histological examination.

The six pups that survived the severe maternal stress (three males and three females) and two nonconstrained controls (1 male and 1 female) were followed until the adult age.

The six animals of the experimental group as well as the two animals of the control group were sacrificed at 1.2 years after their birth. Gross inspection and computerized tomography and histological evaluation were done in all animals.

The experimental protocol was approved by the Animal Research Ethics Committee of the Institution under the protocol 1980-1. The animals were housed in standardized air and light conditions at constant temperature of 22°C with 12-hour light/day cycle. They had free access to tap drinking water and standard laboratory food pellets.

Histologic analysis

Samples of the craniofacial skeleton of each animal were prepared for histologic analysis. The specimens samples were fixed in 10% formalin for 24 hours, decalcified in 5% formic acid for 48 hours, embedded in paraffin, and sectioned at the thickness of 5µm. Sagittal and coronal sections through the cranial base were stained with hematoxylin and eosin to evaluate the premature fusion of the coronal and sagittal sutures as well as the cranial base sutures.

Morphologic measurements

Three distances were standardized based on the calvarial sutures. Two distances transversally and one sagitally.

Transversally: Landmarks for morphologic measurements of the coronal suture were established in the insertion of temporalis muscle. Landmarks for morphologic measurements of the lambdoid suture were established in the bone bridge between temporal bone and occipital bone visible in rats’ calvaria (Figure 2).

Sagitally: Landmarks for sagittal morphologic measurements were established in the imbrications of coronal and lambdoid sutures. The anatomical points were marked with methylene blue and the measurements were performed in 3 consecutive times and averaged (Figure 3).
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FIGURE 3 - The superior view of dissected craniofacial skeleton of adult age rats that had undergone fetal constraint. Note the landmarks for morphologic measurements of the sagittal suture.

The distances were measured using a small caliper.

Computerized tomography

Imaging of the craniofacial skeleton of the Wistar rats were taken at adult age. Rats were sacrificed minutes before the examination. Scans were reconstructed as three-dimensional imaging isosurfaces using InVesalius software, developed by DT3D (Division of Tridimensional Tecnology) - CTI – Brazil. Four distances transversally and two distances sagitally were determined as standards and measured based on well-defined anatomical points.

Transversally: Landmarks for radiologic measurements were established in the union point of zygomatic arch and zygomatic process of the maxilla (ZA-ZPM); the union of coronoid processes of the mandible coinciding with the pterygoid bone in the cranial base (CPM); the union of left and right tympanic cavities (TC); and the union point of zygomatic arch and parietal cranial bones (ZA-PB).

Sagitally: Landmarks for radiologic measurements were established at the union of two points formed by the junction between zygomatic arch and zygomatic process of the maxilla and the junction formed by zygomatic arch and parietal cranial bones. Measurements were taken at left and right sides (LSD; left sagittal distance and RSD; right sagittal distance).

Statistical analysis

The values of the measurements obtained by the CT scans and morphologic measurements were averaged and compared separately to those measurements obtained from the controls using an One-way ANOVA test. The probability value of less than 0.05 was considered as significant. For statistical analysis, we used SPSS V16 software.

Results

Gross findings

The five rats which were born dead had their skull exposed. Visual inspection did not reveal any evidence of synostosis in the coronal, lamboid and sagittal sutures. The six rats which survived the severe maternal stress caused by uterine cerclage had a very small longitudinal size in comparison with their pairs in the control group and relatively shorter upper and lower limbs. Head shape was normal in all of them, with no evidence of synostosis (Figure 4).

Interestingly, during the growth spurt, rats which were offsprings of the constrained group “caught up” to the normal growth level observed in the controls, therefore they had almost the same size of the craniofacial skeleton and weight at adult age. Gross inspection of the craniofacial skeleton did not reveal any alteration of the skull shape, as well as no obliteration of cranial sutures.

We did not observe statistically significant differences in any of the measurements of the craniofacial skeleton taken with the caliper in either group: the female rats or the male rats, both at adult age (p>0.05). All measurements performed with a caliper were shown in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1 - Each anatomic landmark measured with caliper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distances (caliper)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>F.C</td>
</tr>
<tr>
<td>F 1</td>
</tr>
<tr>
<td>F 2</td>
</tr>
<tr>
<td>F 3</td>
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<tr>
<td>M.C</td>
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<tr>
<td>M 1</td>
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<tr>
<td>M 2</td>
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<tr>
<td>M 3</td>
</tr>
</tbody>
</table>

F.C; female control; M.C; male control; F (1-3) = females and M (1-3) = males born after uterine cerclage
**Histological findings**

We did not observe any sign of premature ossification neither in coronal nor sagittal sutures.

**Radiographic findings**

We did not observe statistically significant differences in any of the measurements taken through CT scans (Figure 5 and Table 2).

**TABLE 2** - Each anatomic landmark measured with CAT scan

<table>
<thead>
<tr>
<th>Distances</th>
<th>ZA-ZPM (mm)</th>
<th>CPM (mm)</th>
<th>TC (mm)</th>
<th>ZA-PB (mm)</th>
<th>LSD (mm)</th>
<th>RSD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>11.220</td>
<td>18.105</td>
<td>20.414</td>
<td>17.191</td>
<td>15.720</td>
<td>15.480</td>
</tr>
<tr>
<td>F1</td>
<td>11.421</td>
<td>17.686</td>
<td>16.376</td>
<td>20.100</td>
<td>15.924</td>
<td>15.575</td>
</tr>
<tr>
<td>F2</td>
<td>11.179</td>
<td>17.379</td>
<td>18.894</td>
<td>15.925</td>
<td>15.047</td>
<td>14.516</td>
</tr>
</tbody>
</table>
Distance ZA-ZPM corresponded to the union point of zygomatic arch and zygomatic process of the maxilla (p=0.668); distance CPM corresponded to the union of coronoid processes of the mandible coinciding with the pterygoid bone in the cranial base (p=0.525); distance TC corresponded to the union of left and right tympanic cavities (p=0.164); distance ZA-PB corresponded to the union point of zygomatic arch and parietal cranial bones (p=0.880); distances LSD and RSD corresponded to the union of two points formed by the junction between zygomatic arch and zygomatic process of the maxilla and the junction formed by zygomatic arch and parietal cranial bones at left and right sides (p=0.844) and (p=0.448).

Discussion

No study, which has evaluated the long-term effects of the uterine cerclage on the shape of the craniofacial skeleton in Wistar rat model was found in the current literature.

Investigators have identified abnormal patterns of TGF-ß expression in a model of fetal head constraint. They demonstrated that mechanical forces caused by intrauterine cerclage up-regulates TGF-ß1 in osteoblasts of the involved cranial suture and down-regulates its dural TGF-ß34,6. This phenomenon associated to chondrocyte apoptosis was also seen in the sphenoid-occipital synchondrosis, suggesting morphologic skeletal changes in the cranial base sutures3. These experimental studies corroborate other clinical studies which suggest that non syndromic forms of craniosynostosis might have started in the primary premature fusion of the cranial base sutures7-11. Additionally, it also has been suggested that the premature fusion resulted from a relative fusion of the cranial base sutures8-11. These experimental studies corroborate with this maneuver, where five out of eleven pups were born deliver normally. We significantly decreased the mortality rate 48 hours beyond the normal gestation and we let the mother attempt we had 100% mortality rate in a series of C-sections. Therefore, instead of a C-section, we released the uterine cerclage fetal anoxia and may lead to severe maternal stress. In a preliminary attempt we had 100% mortality rate in a series of C-sections. Hence, we did not test Ihh expression, it may explain the smaller size of upper and lower limbs observed in the Wistar rat. A selective pathway that resulted on impairment of long bones development might have happened.


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