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BRAIN TISSUE ASPIRATION IN NEURAL TUBE DEFECT

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ABSTRACT - The study aimed to find out how frequent is brain tissue aspiration and if brain tissue heterotopia could be found in the lung of human neural tube defect cases. Histological sections of each lobe of both lungs of 22 fetuses and newborn with neural tube defect were immunostained for glial fibrillary acidic protein (GFAP). There were 15 (68.2%) females and 7 (31.8%) males. Age ranged from 18 to 40 weeks of gestation (mean= 31.8). Ten (45.5%) were stillborn, the same newborn, and 2 (9.1%) were abortuses. Diagnosis were: craniorrhachischisis (9 cases, 40.9%), anencephaly (8 cases, 36,4%), ruptured occipital encephalocele and rachischisis (2 cases, 9.1% each), and early amniotic band disruption sequence (1 case, 4.5%). Only one case (4.5%) exhibited GFAP positive cells inside bronchioles and alveoli admixed to epithelial amniotic squames. No heterotopic tissue was observed in the lung interstitium. We concluded that aspiration of brain tissue from the amniotic fluid in neural tube defect cases may happen but it is infrequent and heterotopia was not observed.

KEY WORDS: brain heterotopia, neural tube defect, brain tissue aspiration.

Aspiração de tecido cerebral em casos de defeitos de fechamento do tubo neural

RESUMO - O objetivo do estudo foi identificar qual a freqüência de aspiração de tecido cerebral e a existência de heterotopia nos pulmões de casos humanos de defeito de fechamento do tubo neural através da reação imuno-histoquímica para proteína fibrilar glial ácida (GFAP) em cortes histológicos de todos os lobos de ambos os pulmões de 22 casos de fetos e neonatos com defeito de fechamento do tubo neural. Havia 15 casos femininos (68,2%) e 7 masculinos (31,8%), com idade gestacional variando de 18 a 40 semanas (média= 31,8), sendo natimortos e neomortos 10 (45,5%) cada e 2 (9,1%) abortos. Os diagnósticos foram: Craniorraquisquise (9 casos, 40,9%), anencefalia (8 casos, 36,4%), encefalocele occipital rota e raquisquise (2 casos, 9,1%) e 1 (4,5%)caso de seqüência de disruptura amniótica precoce. Somente 1 caso (4,5%) apresentou células positivas dentro de bronquíolos e alvéolos em meio a células epiteliais amnióticas. Não se observou heterotopia no interstício pulmonar. Concluímos que a aspiração de tecido encefálico do líquido amniótico pode ocorrer em casos de defeito do fechamento do tubo neural, mas são infreqüentes e heterotopia não foi observada.

PALAVRAS-CHAVE: heterotopia cerebral, defeito do fechamento do tubo neural, aspiração de tecido cerebral.

Brain tissue nodules in the lungs are rare and seen generally associated with neural tube defects[1]. Its pathogenesis is controversial and speculative. The two major hypotheses are aspiration of brain fragments after disruption in neural tube defect (NTD) cases[2] and neural tissue migration defects during embryogenesis[3]. Neoplasia[4] and brain tissue embolism[2,5-8] have also been speculated but they do not explain such cases.

The main goal of this study was to determine how often could brain tissue be identified in the bronchial tree and lung parenchyma of NTD fetuses and newborn submitted to postmortem examination with conventional hematoxylin and eosin staining and immunohistochemistry for glial fibrillaryacidic protein (GFAP) in order to determine if aspiration of disrupted brain could explain the occasional finding of brain tissue heterotopia in the lung.

METHOD

We analyzed retrospectively the lungs of 22 fetuses and newborn with NTD submitted to postmortem examination at the Department of Pathology, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil. One section of all lobes of each lung was histo-

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logically processed, embedded in paraffin from which 4 μm sections were examined by light microscopy using hematoxylin and eosin staining and immunohistochemistryfor GFAP with the avidin-streptavidin method. The study was approved by the Ethics Committee of the University Hospital of our institution.

RESULTS

Table shows the clinical data, diagnosis and GFAP result for the 22 cases analyzed. There were 15 (68.2%) females and 17 (31.8%) males. Ten (45.5%) cases were stillborn, 10 (45.5%) cases were early neonatal deaths and 2 (9.1%) were abortuses under 19 weeks of gestation. Of the 10 neonatal death cases, 8 survived less than one hour, one survived 13 hours and another one 2 days. Gestational age ranged from 18 to 40 weeks, with a mean of 31.8 weeks. Craniorrhachischisis was the most prevalent diagnosis, with 9 cases, corresponding to 40.9%, followed by anencephaly, with 8 (36.4%) cases, ruptured occipital encephalocele and rachischisis, with 2 (9.1%) cases each and one case (4.5%) of early amnion band disruption sequence with destruction of the face, calvarium and brain. No brain tissue heterotopia was found in the lung parenchyma in any of the analyzed cases. Only case nr 13, a 28 week-old female stillborn fetus with craniorrhachischisis (4.5%) presented fragments of tissue positive for GFAP inside bronchioles and alveoli admixed to epithelial squames from the aspirated amniotic fluid (Figure). The hematoxylin and eosin stained section of this case showed small

Table. Clinical data, diagnosis and GFAP result of the 22 cases of open neural tube defect analyzed.

Case nr	Gestational age (wks)	Sex	Age	Diagnosis	GFAP
1	40	F	SB	Craniorrhachischisis	negative
2	35	F	3 min	Rachischisis	negative
3	33	M	46 min	Craniorrhachischisis	negative
4	39	F	40 min	Anencephaly	negative
5	38	F	35 min	Anencephaly	negative
6	37	F	SB	Craniorrhachischisis	negative
7	35	F	SB	Anencephaly	negative
8	19	F	Ab	Craniorrhachischisis + diaphragmatic eventration	negative
9	37	M	2 d	Anencephaly	negative
10	34	F	3 min	Lumbar rachischisis	negative
11	40	F	13 h	Anencephaly	negative
12	27	M	SB	Anencephaly	negative
13	28	F	SB	Craniorrhachischisis	positive
14	18	M	Ab	Amniotic band disruption sequence	negative
15	28	M	SB	Craniorrhachischisis	negative
16	26	M	SB	Ruptured occipital encephalocele	negative
17	38	M	SB	Ruptured occipital encephalocele	negative
18	36	F	39 min	Anencephaly	negative
19	31	F	SB	Craniorrhachischisis	negtive
20	30	F	SB	Anencephaly	negative
21	21	F	50 min	Craniorrhachischisis	negative
22	30	F	41 min	Craniorrhachischisis + iniencephaly	negative

F, femine; M, masculine; SB, stillbirth; Ab, abortus; min, minutes; h, hours; d, days.

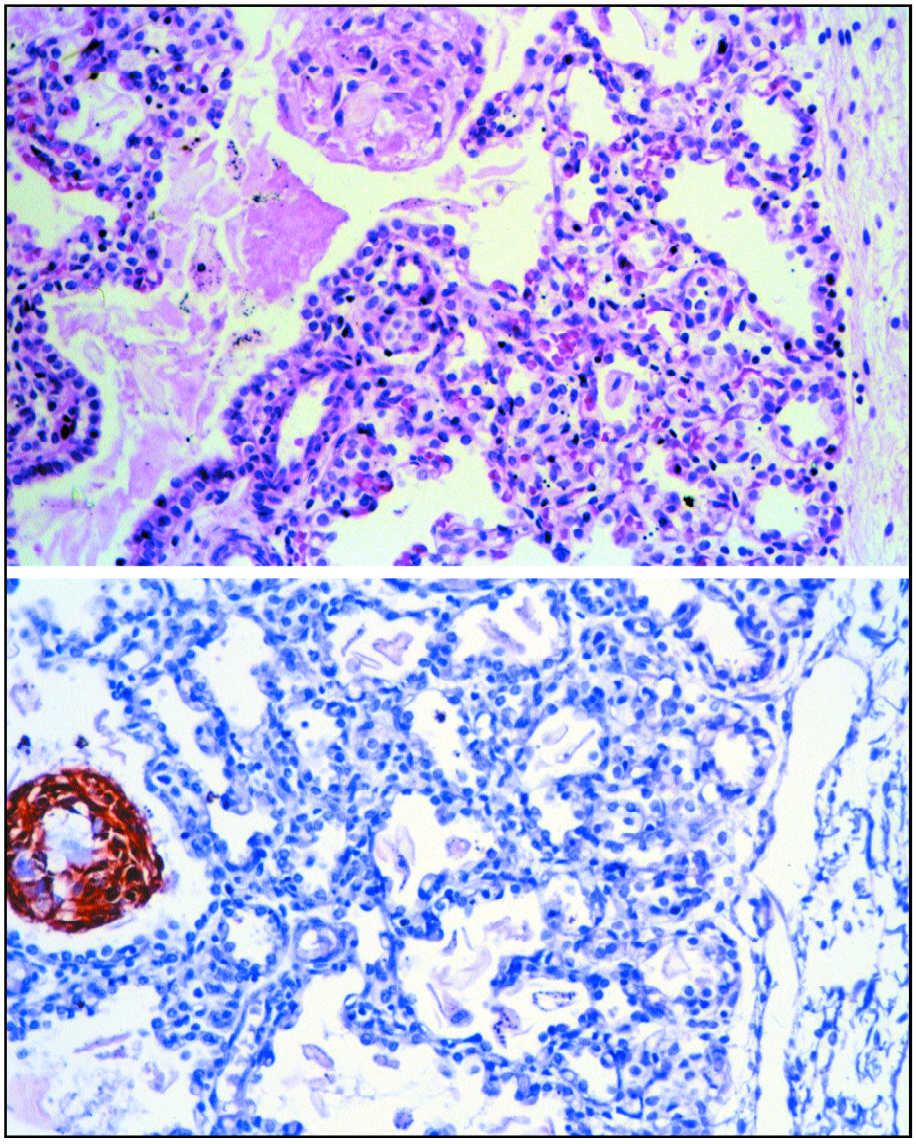

Figure. Photomicrographs of the lung in which GFAP positive cells were identified. Note that in the hematoxylin and eosin stained section (top) the aspirated material do not reveal the true nature of the cells that are clearly identified with the immunostaining for GFAP (bot - tom). (Top: hematoxylin and eosin, 400X; Bottom: immunostaining for GFAP, 400X).

intraluminal masses of cells with round to oval nuclei and flat squames immersed in an amorphous background that did not present recognizable glial and neuropil features. Bronchial cartilage was also positive for GFAP in some cases.

DISCUSSION

Brain tissue heterotopia is a rare lesion and this fact has influenced our understanding of its pathogenesis. In the beginning of the 20th century it was interpreted as a neoplasia[4] latter discarded[9]. Embolism of brain tissue may be seen after traumatic breech delivery[7] and trauma to the head[8] in any age. The brain tissue in this instance is found inside blood vessels and death occurs shortly leaving no time for implantation, if it was possible at all. Aspiration of brain fragments[2,5-8] and modeling or migration defects are the most likely explanations[10,11].

In this study, only one case in 22 (4.5%) showed brain tissue inside the bronchioles and alveoli. This tissue was admixed to the common epithelial squames from the amniotic fluid forming small masses. Its true nature was clearly depicted by the immunostaining for GFAP and would possibly have been missed if only hematoxylin and eosin stained sections have been used. Neuron specific enolase

has been used to show neuronal differentiation[1] and other antibodies such as neurofilament and synaptophysin may also be used. In fact, the routine use of immunohistochemestry in the lungs of cases presenting open NTD would allow the diagnosis of both aspiration, as seen in the present study, and interstitial implantation[1].

There was no implantation or growing of the brain tissue but its presence indicates that aspiration may well be a mechanism that makes it possible. Anyway, the cases studied here had a gestational age higher than that expected for implantation to arise since the chance of brain tissue disruption is higher in early gestation. However, respiratory movements are fewer and the lungs exhibit only budding bronchi by the time brain fragments could be found more often in the amniotic fluid.

Brain fragments in the amniotic fluid are well documented in the literature with the transformation of early exencephaly to late anencephaly in animal models[12]. These fragments could surely be inhaled as well as swallowed by the fetus since respiratory movements and swallowing is present as early as 12 weeks of gestation[13]. Implantation of such fragments would require viable brain fragments and an adequate implantation surface. Soon after disruption at least part of the brain tissue fragments may well be viable. As the finding of brain tissue heterotopia is rare implantation is probably the limiting part of the process.

Swallowing of amniotic fluid is physiological and so brain fragments present in it could theoretically implant over the gastrointestinal mucosa and yet there is not a single report of its occurrence, maybe indicating that there are no favorable conditions for its implantation. Is the airway mucosa different? In case there are special conditions for its implantation, differently from other sites, why is it so rare? These questions are still unanswered demanding further studies.

An argument favoring implantation is its occurrence with ruptured teratomas, when glial elements may spread over and grow on the peritoneum[14]. In fact, there is a report of brain tissue implants over the abdominal surface of the diaphragm in a case of NTD and gastroschisis[15] and more recently appeared a report on a fatal case of multiple brain tissue heterotopia in an otherwise normal baby, product of monochorionic monoamniotic gestation in which his twin brother presented anencephaly[16].

Whereas, some cases reported in the literature indicate that aspiration may not be the only mechanism. The finding of brain nodules in the lung in cases with no open NTD[10,17] may favor other mechanisms such as modeling or migration defect.

In conclusion, the data presented in this study show that aspiration of brain tissue fragments do occur and favors the aspiration hypothesis although we could not find evidences of viable tissue in order to implant and grow and also the aspirated fragments were inside airspaces not in the interstitium. Since there is yet no animal model for brain tissue heterotopia and human cases are rare other mechanisms such as a modeling or migration defect must not be discarded.

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