Bilateral Neuroblastoma in Situ associated with Microcephaly

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We present an autopsy case of a two-day-old female infant with a very unusual combination of neuroblastoma in situ in both adrenals and microcephaly. This baby was born to a 28-year-old mother after 38 weeks of gestation, and died of respiratory difficulty 2 days later. At autopsy, the baby weighed 1,840gm, and the brain was extraordinarily small with a weight of 125gm. The gyral pattern was simplified and irregular. Microscopically massive migration defects, pachygyria, micropolygyria, leptomeningeal glioneuronal islands, small corticospinal tract and heterotopic Purkinje cells in the cerebellum were found. In addition, there were medullary nodules in both adrenals. They measured $0.7 \times 0.4\text{cm}$ and $0.7 \times 0.3\text{cm}$, respectively. These nodules showed the typical histological features of undifferentiated neuroblastoma. The tumor nodules were confined to the medullary portion and did not extend to the cortex or contiguous structures meeting the criteria of neuroblastoma in situ. Based on these unusual and seemingly unrelated sets of findings, it is suggested that the histogenesis of neuroblastoma in situ could be a part of the generalized dysontogenic process.

Key Words: Neuroblastoma, Neuroblastoma in situ, Microcephaly

INTRODUCTION

Neuroblastoma is one of the most common malignant solid tumor in young children (Dehner, 1987; Berry, 1987). The tumor cells are thought to arise from primitive sympathetic neuroblasts of neural crest origin. Congenital neuroblastoma undergoes spontaneous regression more commonly than any other tumor. This is usually the result of necrobiosis, but maturation to ganglioneuroma may occur (Berry, 1987). Small lesions in the adrenal medulla cytologically resembling typical malignant neuroblastoma had been reported even in old literature (Morison, 1952). Most of them were not considered to be true tumors. In 1963, Beckwith and Perrin reported 13 cases of neuroblastoma in situ identified at autopsy; in a review of

the literature they found incomplete reports of 7 other similar lesions (Beckwith et al., 1963). They considered the lesions as malignant tumors and estimated the true incidence of neuroblastoma in situ to be about 40 times greater than the number of cases of clinically diagnosed disease. In 1940, Wells included this lesion in his series of congenital malignant neoplasms. However, he doubted as to its actual nature and proposed that it was not in fact a true tumor, but merely an abnormal accumulation of undifferentiated sympatheticoblasts which might be present not rarely in fetus (Wells, 1940).

This communication deals with a case of neuroblastoma in situ that was accompanied by microcephaly resulting from the defective process of cellular migration during the developmental period.

METHODS

The autopsy was done according to routine procedure, and the dissected organs or tissues were fixed in neutral formaldehyde solution. The histologic speci-

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mens were prepared according to routine procedure and embedded in paraffin. Routine hematoxylin-eosin staining was done. Using the micrometer eyepiece at 1,000 times magnification the nuclei of the tumor cells were measured. Immunohistochemical staining of neuron-specific enolase (Dako Co., Santa Barbara, Calif.) was performed on the paraffin sections by the peroxidase-antiperoxidase method. Chromosome study was not done.

DNA flow cytometry was performed using samples prepared from archival paraffin blocks. The method for preparation was slightly modified from that of Hedley et al (1983). Briefly 50 μ m sections were dewaxed after removing the adrenal cortex, rehydrated and incubated in pepsin for 30 min at pH1.5. The resulting suspension was centrifuged and the pellet resuspended in isoton containing 1 μ g/ml of 4, 6-diamidino-2-phenylindol-hydrochloride. The suspension was then syringed through a narrow gauge needle and filtered through a 35 μ m pore size nylon gauze. Approximately 10,000 cells per sample were analysed on a FACScan (Becton-Dickinson, USA, Calif.). For negative control, peripheral lymphocytes of normal human were used.

REPORT OF A CASE

This female infant was born to a 28-year-old mother after 38 weeks of gestation. The mother had experienced two spontaneous and one therapeutic abortions, and had no children. The pregnancy was uneventful until 38 weeks, when ultrasonography disclosed a fetal anomaly i.e. microcephaly. Delivery was induced and the patient died 2 days later. During two days of life, she suffered from dyspnea.

The fetal measurements were as follows; body weight, 1840gm; crown-rump length, 27cm; crown-heel length, 39.5cm; and left foot length, 6.2cm. The head, chest, and abdominal circumferences were 26, 26, and 24cm, respectively. The fetus was small for the gestational age, but no external gross anomaly was noted. A radiograph of the head showed a sloping forehead and a small calvarium, particularly compared to the face (Fig. 1). Bilateral incomplete lobation of the lung and large patent ductus arteriosus were noted. No other internal anomalies were noted, except for slightly enlarged adrenals. The weight of the brain was 125gm. The antero-posterior and biparietal diameters of the brain were 6.8cm and 7.3cm, respectively. The parietal lobe was underdeveloped and the occipital lobe was tracted upward, so it appeared as if the occipital lobe was underdeveloped (Fig. 2). The gyral pattern was distorted in many areas, showing inter-

Fig. 1. Radiograph of the skull in lateral view. Sloping forehead and small calvarium are seen.

Fig. 2. Lateral view of the brain. The parietal lobe is underdeveloped and the occipital lobe is retracted upward. It appears that the occipital lobe was underdeveloped. Leptomeningeal opacity is also noted.

ruption, irregular thickening or granularity. The cerebellum was not completely covered by the occipital lobe. The leptomeninges were slightly opaque. The adrenals were cut after fixation and there were graywhite ill-defined nodules in the medullary portions. The right and left nodules measured 0.7 $\times$ 0.4cm and 0.7 $\times$ 0.3cm, respectively (Fig. 3).

Histologically, pachygyria, micropolygyria, and leptomeningeal glioneuronal islands were found in the cerebrum (Fig. 4). Nests of heterotopic Purkinje cells were seen near the dentate nucleus of the cerebellum. The pyramis was small and so was the spinal cord. Meanwhile the adrenal nodules were composed of a dense accumulation of undifferentiated neuroblastic cells. The cells were ovoid and small, measuring less than 5 μ m. The cytoplasm was scanty and the

Fig. 3. Bilateral small nodules are seen in the cut surface of both adrenals (a: right, b: left). Arrow heads show borders of nodules in the adrenal medulla.

Fig. 4. Microscopic view of the brain. Micropolygria and heterotopic leptomeningeal glioneuronal islands (arrow) are seen (hematoxylin-eosin, x40).

nucleus was hyperchromatic (Fig. 5). Neither necrosis nor hemorrhage was found in the nodules, and calcification was not seen. Homer-wright rosettes were not seen. Mitotic figure was seldom seen. The neuroblastic nodules were confined to the medulla in both adrenals, and did not infiltrate into cortex. The composing cells showed strong positivity for neuron-

Fig. 5. Microscopic view of undifferentiated neuroblast cells in the right adrenal. Immunoperoxidase stain for neuron-specific enolase demonstrates strong positive reaction (Insert). (hematoxylin-eosin, x400, and neuron-specific enolase, x400)

specific enolase (Fig. 5). Metastatic focus was not identified in the remaining organs or tissues. On DNA flow cytometry, the right and left tumor cells were all diploidy (Fig. 6), but the percents of in G2 + M phase were 7.8% and 12.8% in right and left respectively. The nuclei of tumor cells were $5.40 \pm 0.70 \mu\text{m}$ (right) and $5.15 \pm 0.88 \mu\text{m}$ (left) in diameter.

DISCUSSION

Our case had two important congenital anomalies i.e. bilateral neuroblastoma in situ and microcephaly. The nature of the neuroblastoma in situ has not been clearly elucidated whether these neuroblastic nodules are only variations of normal development or are related to malignant neuroblastoma.

Beckwith and Perrin described this small nodule in the adrenals of fetal and neonatal autopsy as "in situ neuroblastoma" (Berry, 1987). They hypothesized that this lesion could become malignant neuroblastoma. The nodule described by Beckwith and Perrin was $60 \mu\text{m}$ and on average was less than $400 \mu\text{m}$. The criteria of neuroblastoma in situ by Beckwith and Perrin were as follows; 1. the lesions are distinct tumor nodules, composed of immature neural crest elements indistinguishable from those of typical malignant neuroblastoma, 2. adequate sections demonstrating the lesion are available, 3. there is neither gross nor microscopic evidence of tumor elsewhere in the body, and 4. the lesion is presumably detected only with the microscope (Beckwith et al., 1963). The lesion in our case is compatible with these criteria. The lesions

Fig. 6. DNA flow cytometric analysis of tumor cells (a. negative control, b. right, c. left). The cells in G2 + M phase (right, 7.8%; left, 12.8%) are higher than that of negative control, but there is no evidence of aneuploidy.

in the cases of Beckwith and Perrin range from 0.7mm to 9.5mm and are associated with several congenital anomalies. Seven out of 13 cases of Beckwith and Perrin had cardiovascular anomalies, and 2 cases had hydrocephalus and Arnold-Chiari malformation. In our case, both cardiovascular anomaly (patent ductus arteriosus) and microcephaly were associated, although associated anomalies are of a different variety. Several investigators argued that neuroblastoma in situ is a part of a generalized dysontogenetic process. Meanwhile, Wells included this lesion in his series of congenital malignant neoplasms (Wells, 1940). Reviewing the literature including 1,500 cases of neonatal autopsy Morison described the microscopic size of neuroblastic nodules as simple undifferentiated cell clusters rather than malignancy. Simple microscopic clusters of neuroblastic cells are frequently found in the fetal adrenal gland (Turkel et al., 1974; Chi et al., Ikeda et al., 1981). The average incidence is 37.2% and maximal incidence is 54% in the fetal adrenal glands between 16 and 20 weeks (Chi et al., 1980). Therefore, these simple cell clusters should be distinguished from a sizable nodular mass, i.e., neuroblastoma in situ.

DNA aneuploidy was frequently found in neuroblastoma (Naito et al., 1991). These both tumors were diploid. The nuclear size of tumor cells was correspond to primitive sympathetic neuroblasts (Ikeda et al., 1981).

In recent years the term microcephaly has been assigned to persons whose head circumference is smaller than 3 standard deviation (SD) below the mean for age and sex (Warkany et al., 1981), but in some studies deviations of 2 SD from the mean have also been included. The head circumference of our case is 26cm and average body measurements of Korean fetus in relation to body weight are as follows: body weight, 1850gm (SD 66.5gm), crown-rump length, 28.8cm (SD 1.7cm), head circumference, 29.8cm (SD

2.3cm), and brain weight, 263gm (SD 28.2gm) (Chi et al., 1989). This baby showed generalized underdevelopment, so the head circumference and brain weight should be based on the age and sex of this fetus. The brain in this case weight 125gm, indicating a severe reduction in relation to the head circumference. Microcephaly is classified into primary (genetic) or secondary (environmental): gene mutation, chromosomal damage or environmental damage (Warkany et al., 1981); or genetic, environmental or unknown etiology (Ross et al., 1977). Multiple anomalies associated with microcephaly have been reported i.e. Down syndrome, trisomy 13 syndrome, trisomy 18 syndrome, Bloom syndrome, and so on. However, there is no report of neuroblastoma in situ associated with microcephaly. In addition to a small size of the brain, microcephalic brains are known to show diverse changes which include gliotic encephalopathy, prosencephaly, porencephaly, arhinencephaly, system atrophy, or evidence of disorderly cell migration (Larroche, 1977). The pathologic features of being distorted cell migration in the central nervous system include micropolygyria, pachygyria, status verrucosus and heterotopias. Among these, micropolygyria, pachygyria, and heterotopia were seen in this case, indicating that the microcephaly in this case could be the result of massive cell migration defect.

There have been several reports about neuroblastoma in situ in the literature since Beckwith and Perrin in 1963 (Fryns et al., 1982; Guin et al. 1969; Hasegawa et al., 1982; Tubergen et al., 1970). Examining all the unusual features in our case one would like to assume that the pathogenesis of neuroblastoma in situ may be dysgenesis of cell migration of primitive neuroblast from neural crest which could also affect the migration of cells in the developing central nervous system. However, the possibility that this association is coincidental should be considered.

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