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Original Article

Case-control study of perinatal factors and hepatoblastoma in children with an extremely low birthweight

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Abstract

Background: There is a significant association between hepatoblastoma and low birthweight. A case-control study was conducted to reveal risk factors for hepatoblastoma in children of extremely low birthweight (< 1000 g).

Methods: Prenatal and postnatal histories, including parental histories, of 12 hepatoblastoma cases and 75 birthweight-matched controls were compared.

Results: The gestational age of the hepatoblastoma cases (23–32 weeks; median 25 weeks), tended to be lower than that of the controls (23–36 weeks; median, 27 weeks; $P = 0.072$). The time for an infant's bodyweight to return to the same level as the birthweight also tended to be longer in hepatoblastoma cases than in controls ($P = 0.055$). All hepatoblastoma cases received oxygen therapy for a period of 4–508 days. (median 114 days), which was significantly longer than the 0–366 days (median 62 days) in the controls ($P = 0.022$). Furosemide was given to all hepatoblastoma cases and was used for a significantly longer period in these infants (6–460 days; median 89 days) than in the controls (0–241 days; median 44 days $P = 0.027$). A univariate Cox regression demonstrated that the time taken to regain bodyweight at birth and the duration of both oxygen therapy and furosemide treatment were significantly associated with the development of hepatoblastoma (hazard ratio (HR) = 1.044, $P = 0.013$; HR = 1.006, $P = 0.001$; and HR = 1.007, $P = 0.001$, respectively). Although there were significant correlations between the factors, a multivariate Cox regression analysis identified the duration of oxygen therapy as a significant independent risk factor (HR = 1.006, $P = 0.001$).

Conclusions: Oxygen therapy and furosemide treatment, along with the rate of growth, are risk factors for the development of hepatoblastoma in children of extremely low birthweight, and the duration of oxygen therapy is the most important factor in predicting the development of hepatoblastoma. Further studies are necessary to determine the real reasons for the development of hepatoblastoma and to protect children of low birthweight from the development of cancer.

Key words extremely low birthweight, furosemide, hepatoblastoma, oxygen.

Hepatoblastoma is the most common malignant hepatic tumor in children and, presumably, develops in the liver undergoing disturbed organogenesis. More than 80% of tumors occur in children under 3 years of age. Some hepatoblastomas are associated with congenital abnormalities, such as Beckwith–Wiedemann syndrome, hemihypertrophy, trisomy 18 syndrome and familial adenomatous polyposis, and identification of the genes responsible for these abnormalities and hepatoblastoma has been a focus of research.[1–3] However, the majority of hepatoblastomas are sporadic cases that appear to be unrelated to the genetic predisposition. Although little is known about the etiologies of the tumor, parental occupational exposure to metals, petroleum products and paints or pigments is reported to be a risk factor for the development of hepatoblastoma.[4]

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Epidemiological studies that analyzed data in the Japan Children's Cancer Registry have revealed that there is a significant association between hepatoblastoma and low birthweight. The incidence of hepatoblastoma in children of very low birthweight (< 1500 g) is increasing in Japan.[5] It also was demonstrated that the lower the birthweight, the higher the incidence of hepatoblastoma.[6] Follow-up studies in the US confirmed this association, which implied that the phenomenon may be widespread.[7,8] At the same time, these observations suggest that genetic or environmental factors may be responsible for hepatoblastomas in children of very low birthweight. However, there was no difference between patients of low birthweight and other patients in age at diagnosis of hepatoblastoma.[6] Birth prevalence rates of congenital anomalies or light-for-date infants were similar in the two groups of patients.[6] Subsequent analyses showed that the patient's prematurity, the intensive treatments due to neonatal illnesses and the development of advanced hepatoblastoma with unfavorable biological behavior were closely correlated.[9,10] Because these findings strongly suggest the presence of some environmental factors responsible for the development of hepatoblastoma, we conducted a case-control study and compared both parental histories and patients' perinatal histories with those of controls. In the present paper, we report the results of a multicenter study of risk factors for hepatoblastoma in children of extremely low birthweight (< 1000 g).

Methods

At the time of this study (November 1997), 13 hepatoblastoma patients of very low birthweight (< 1500 g) had been identified from the registry data of the Japan Children's Cancer Registry. These patients were among the 301 hepatoblastoma patients who were diagnosed during the 11 years between 1985 and 1995. Two additional patients, who had been presented in Japanese publications during the same period, were identified from the data base of medical journals. The characteristics of these 15 patients, including disease stage, treatment and outcome as well as parents' histories and patients' prenatal and postnatal histories, were analysed and have been described in detail in previous publications.[9,10]

Three patients with a birthweight of between 1000 and 1500 g were excluded from this study on the assumption that this exclusion would make the cases pathogenetically homogeneous and, thus, the cases studied consisted of 12 hepatoblastoma patients of extremely low birthweight (< 1000 g). Patients were born between 1988 and 1994 and were diagnosed as having hepatoblastoma at ages ranging from 6 to 77 months (median 16 months). During the neonatal period, they were referred to their regional secondary or tertiary neonatal centers or neonatal intensive care units. Because two cases were treated at the same hospital and another two were treated at the same neonatal intensive care unit, a total of 10 hospitals were involved in the treatment of postnatal illnesses of these 12 cases.

Controls were collected from three of 10 hospitals. These hospitals (Maternal and Perinatal Center of Tokyo Women's Medical College, Department of Neonatology at Osaka Medical Center for Maternal and Child Health and Department of Neonatology at Gunma Children's Medical Center) treat a relatively large number of infants and so controls were able to be collected without difficulty. Twenty-five infants born between 1988 and 1994 with a birthweight < 1000 g were randomly selected from the administrative registry of each hospital without any further specific conditions applied and a total of 75 birthweight-matched controls were collected. At the time of this analysis, the controls had been followed up for 1068–3505 days after birth and no hepatoblastoma had been observed in any of them.

The medical records of mothers and infants were reviewed in a blinded fashion by each attending neonatologist without knowing further analytical methods. A variety of factors relating to pre- and postnatal histories of the cases and controls were compared. Maternal (parental) and prenatal factors analysed included family history, occupation, reproductive history, alcohol intake or cigarette smoking during pregnancy, pre-eclampsia, prolonged rupture of membrane (≥24 h), prenatal infection, intra-uterine growth retardation, corticosteroid administration or other medication during pregnancy, fetal asphyxia, maternal age at delivery and mode of delivery. Postnatal factors were gestational age, gender, growth in terms of bodyweight gain, congenital abnormalities, serum IgM level at birth and early postnatal illnesses, such as respiratory distress syndrome, symptomatic patent ductus arteriosus, chronic lung disease, sepsis, liver dysfunction and biliary disorders. Treatments and medications, as well as radiological examinations given at a neonatal intensive care unit, were also examined. Treatments reviewed in the present study included oxygen therapy, assisted ventilation and phototherapy. The investigation covered 12 medicines that had already been confirmed to have been used in more than four of 15 hepatoblastoma patients of very low birthweight (< 1500 g) and these were furosemide, aminophylline, spironolactone, dopamine, alphacalcidol, ampicillin, cefmetazole, dexamethasone, phenobarbital, dobutamine, mefenamic acid and lung surfactant.[10]

Data are shown as the distribution range and the median value. Mann–Whitney's *U*-test was used to compare continuous variables. Differences between two groups in categorical data were analyzed by Fisher's exact probability test or the Chi-squared test. Because the period of

Table 1 Maternal reproductive history and neonatal characteristics

	Range (median)		*P*
	Cases ($n = 12$)	Controls ($n = 75$)	
Previous pregnancy	0–3 (1)	0–5 (1)	0.590
Parity	0–2 (1)	0–3 (0)	0.383
Spontaneous abortion	0–2 (0)	0–2 (0)	0.977
Maternal age at delivery (years)	23–39 (30)	18–42 (30)	0.795
Gestational age (weeks)	23–32 (25)	23–36 (27)	0.072
Birthweight (g)	560–974 (807)	507–999 (816)	0.413
Gender (male/female)	7/5	32/43	0.360
Time to regain bodyweight at birth (days)	18–77 (33)	1–59 (28)	0.055
Time to reach 2000 g bodyweight (days)	47–252 (129)	48–289 (99)*	0.076

*Data were available in 74 controls.

observation was different for each case and control, Cox regression models were used to explore univariate associations between potential risk factors and the development of hepatoblastoma. The multivariate Cox regression analysis by the backward stepwise (Wald) method was performed in order to identify an independent predictor of hepatoblastoma after controlling other risk factors. Pearson's correlation coefficient was calculated to examine correlations between the risk factors. Statistical analysis was performed with SPSS 7.5J for Windows Medical Pack (SPSS Inc., Chicago, IL, USA) and statistical significance was declared at $P < 0.05$.

Results

Maternal reproductive history and neonatal characteristics

Maternal reproductive history and neonatal characteristics in children of extremely low birthweight with or without hepatoblastoma are summarized in Table 1. There were no significant differences between the cases and controls with regard to factors relating to maternal reproductive history: the numbers of previous pregnancies, parity and spontaneous abortions and the age at delivery. Family history, parents' occupation and histories regarding alcohol abuse and cigarette smoking during pregnancy in the groups were not significantly different (data are not shown). Although the birthweight was completely matched between cases and controls ($P = 0.413$), the gestational age of the cases (23–32 weeks; median 25 weeks) tended to be lower than that of the controls (23–36 weeks; median 27 weeks; $P = 0.072$). The period in which an infant's bodyweight returned to birthweight was longer in cases than in controls and it also took longer for cases to reach to a bodyweight of 2000 g compared with controls, although these differences were not statistically significant ($P = 0.055$ and 0.076, respectively).

Pre- and postnatal histories

With regard to pre- and postnatal histories, there was no history that was significantly associated with hepatoblastoma (Table 2). Whereas 17 (23%) controls received prenatal administration of corticosteroids to stimulate lung maturation, none of the cases was treated antenatally with corticosteroids. An atresia of the external auditory canal and ventricular septal defect were congenital anomalies seen in the cases of hepatoblastoma. Congenital abnormalities seen in four children in the control group were atrial septal defect, ventricular septal defect, congenital hypothyroidism and renal tubular acidosis. The presence of congenital abnormalities was not a factor associated with the development of hepatoblastoma. Neither high serum IgM at birth (≥20 mg/dL) nor early postnatal illnesses, including liver dysfunction defined as high (> 100 IU/L) serum glutamic pyruvic transaminase (GPT) levels, were particular events specific to the cases.

Treatments, medications and radiological examinations in neonatal intensive care units

With regard to treatments, medications and radiological examinations in neonatal intensive care units, the length of oxygen therapy and furosemide treatment in the cases and controls was significantly different (Table 3). Oxygen therapy was given to all cases for a period of 4–508 days (median 114 days), which was significantly longer than the 0–366 days (median 62 days) for controls (69 of 75 infants in the control group received oxygen therapy; $P = 0.022$). Of the 12 medicines investigated in the present study, furosemide was the only medicine that was given to all cases and was used for a significantly longer period in cases than in controls. Cases were treated with furosemide for 6–460 days (median 89 days), but the controls were treated with furosemide for 0–241 days (median 44 days; 57 of 75 control infants received furosemide treatment; $P = 0.027$).

Table 2 Pre- and postnatal histories and hepatoblastoma

Pre- and postnatal histories	Frequency		OR (95% CI)
	Cases	Controls	
Prenatal			
Asphyxia	6/10	36/71	1.458 (0.379–5.614)
IUGR	4/12	22/75	1.205 (0.329–4.416)
Prolonged rupture of membrane (≥24 h)	3/12	17/75	1.137 (0.277–4.677)
Cesarean section	6/12	52/75	0.442 (0.129–1.518)
Pre-eclampsia	1/12	19/74	0.263 (0.032–2.176)
Antenatal corticosteroids	0/12	17/74	–
Postnatal			
High serum IgM at birth (≥ 20 mg/dL)	3/8	8/58	3.750 (0.746–18.840)
Congenital abnormalities	2/12	4/75	3.550 (0.574–21.952)
Liver dysfunction (GPT ≥100 IU/L)	1/12	3/75	2.182 (0.208–22.887)
Cholelithiasis	1/12	3/75	2.182 (0.208–22.887)
RDS	5/12	39/75	0.659 (0.192–2.264)
CLD	6/12	50/75	0.500 (0.146–1.709)
Sepsis	1/12	12/75	0.477 (0.056–4.049)
Symptomatic PDA	2/12	29/75	0.317 (0.065–1.552)

CI, confidence interval; IUGR, intra-uterine growth retardation; GPT, glutamic pyruvic transaminase; RDS, respiratory distress syndrome; CLD, chronic lung disease; PDA, patent ductus arteriosus.

Table 3 Treatments, medications and radiological examinations in neonatal intensive care units

	Range (median)		*P*
	Cases ($n = 12$)	Controls ($n = 75$)	
Treatments (days)			
Oxygen therapy	4–508 (114)	0–366 (62)	0.022
Assisted ventilation	10–295 (64)	0–255 (36)	0.306
Phototherapy	0–27 (7)	1–21 (7)	0.566
Blood transfusion*	0–44 (4)	0–34 (3)	0.492
Blood derivatives*	0–12 (1)	0–13 (1)	0.208
Medications† (days)			
Furosemide	6–460 (89)	0–241 (44)	0.027
Aminophylline	0–118 (22)	0–290 (38)	0.200
Spironolactone	0–352 (23)	0–241 (0)	0.224
Dopamine	0–95 (3)	0–80 (4)	0.770
No. radiological examinations			
Plain chest and abdominal X-rays	4–122 (26)	2–70 (23)	0.690

*The data here are expressed as frequency (times) instead of length (days). †Medicines used in more than six cases are listed.

The duration of assisted ventilation and phototherapy for neonatal hyperbilirubinemia and the frequencies of blood transfusion and blood derivatives, such as albumin, for the two groups were not significantly different. The number of plain chest and abdominal X-rays was similar in both groups.

Clinical characteristics and risk of hepatoblastoma

Univariate associations between the potential risk factors and hepatoblastoma were assessed with the Cox regression models (Table 4). The period to regain bodyweight at birth and the duration of both oxygen therapy and furosemide treatment were significantly associated with the development of hepatoblastoma (hazard ratio (HR) = 1.044, $P = 0.013$; HR = 1.006, $P = 0.001$; and HR = 1.007, $P = 0.001$, respectively) and the proportional hazard was confirmed in these factors after classifying them into four or five categories. The gestational age and the number of plain chest and abdominal X-rays were not associated with the development of hepatoblastoma. There was a significant correlation between the duration of oxygen therapy and furosemide treatment (correlation coefficient = 0.720; $P < 0.001$). In addition, the period to regain birthweight was significantly correlated with the duration of oxygen therapy (correlation coefficient = 0.297; $P = 0.005$) and furosemide treatment (correlation coefficient = 0.293; $P = 0.006$). Multivariate Cox regression analysis by the backward stepwise (Wald) method identified the duration of oxygen therapy as a significant independent risk factor for the development of hepatoblastoma (HR = 1.006; $P = 0.001$).

Discussion

In Japan, a trend towards an increase in hepatoblastoma in children of very low birthweight has become evident since

Table 4 Clinical characteristics and risk of hepatoblastoma*

Characteristics	Hazard ratio (95% CI)	*P*
Regaining bodyweight at birth (days)	1.044 (1.009–1.081)	0.013
No. plain chest and abdominal X-rays	1.020 (0.997–1.044)	0.095
Furosemide treatment (days)	1.007 (1.003–1.011)	0.001
Oxygen therapy (days)	1.006 (1.003–1.010)	0.001
Gestational age (weeks)	0.801 (0.600–1.069)	0.131

*Univariate analysis with the Cox regression model.
CI, confidence interval.

1990.[5] The trend was specifically observed in hepatoblastoma, which suggests that there are some reasons for the trend other than the simple increase in the number of premature infants who survive. Because subsequent studies clarified patient characteristics, the presence of genetic vulnerability or predisposition to hepatoblastoma is less likely to be the underlying factor for the development of hepatoblastoma in these children.[6,9,10] Surgical and pathologic reviews showed that hepatoblastomas were at more advanced stages in children of very low birthweight than in other patients and that tumors at advanced stages occured in extremely premature infants whose gestational age was 23–25 weeks.[9] When perinatal factors for patients with resectable disease (stages II and IIIA) and those with unresectable disease (stages IIIB and IV) were compared, the duration of oxygen therapy and furosemide treatment was significantly longer in patients in the latter than the former group.[10] These findings led to speculation that an unfavorable hepatoblastoma developed when liver cells were exposed to an environmental etiologic factor at an earlier stage of organogenesis. These cells may transform into tumor cells with more unfavorable characteristics. Another hypothesis was that an unfavorable tumor arose in a transformed liver cell in which more genetic damage accumulated due to exposure to a larger quantity (as a result of long-term exposure) of a toxic agent. Whatever the precise mechanisms were, it seemed that all these results were in favor of the presence of an environmental etiology rather than a genetic predisposition to the tumor.

In the present case-control study, both parental and patient histories were compared with birthweight-matched controls and their parents. The study shows that the period to regain bodyweight at birth and the duration of oxygen therapy and furosemide treatment were significantly associated with the development of hepatoblastoma and the duration of oxygen therapy was the most important factor in predicting the later development of hepatoblastoma. The HR for oxygen therapy was 1.006, which means that the risk of developing hepatoblastoma increases by 0.6% when oxygen is given for 1 day. The risk increases by 20% when oxygen therapy is continued for 30 days and increases by 100% in children who are treated with oxygen for 4 months. As mentioned above, a possible relationship between neonatal intensive treatment and hepatoblastoma has already been shown in a previous study[10] and, again, the present case-control study identified oxygen therapy and furosemide treatment as factors significantly associated with the development of hepatoblastoma. Similar significant results were obtained when statistical analyses were performed with controls selected from the 75 control subjects to adjust both birthweight and gestational age (data not shown because of the biased analyses), so the association between intensive treatment and development of hepatoblastoma seems to be essential. In contrast, the period to regain bodyweight at birth usually reflects the severity of illnesses treated at neonatal intensive care units. At the same time, this may be an indirect factor related to furosemide treatment, because infants who are treated with diuretics usually gain bodyweight more slowly than those who are not treated with furosemide. Therefore, it is easy to believe that more intensive treatments would have been administered to infants who gained bodyweight slowly than to those who caught up their bodyweight adequately.

Although there remains the possibility that intensive treatment with oxygen and furosemide is a surrogate for some other causal factor, such as severity of the illness in the neonates, the results suggest a possible role for the treatments themselves in hepatoblastoma tumorigenesis. In recent years, there has been accumulating evidence that oxygen free radicals are involved in the process of illnesses of prematurity, such as chronic lung disease, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhage.[11–13] Excess oxygen induces a greater production of oxygen free radicals, which, together with lung immaturity, barotrauma and secondary neutrophile infiltration, causes neonatal lung injury. Whereas retinopathy of prematurity is an oxidative injury caused primarily by a high oxygen concentration, necrotizing enterocolitis and intraventricular hemorrhage in the newborn result from excess radical formation stimulated by re-oxygenation after hypoxia. The defense mechanism against oxygen free radical damage is immature in premature infants and the low antioxidant activity is associated with mortality of infants as well as the development of the illnesses of prematurity.[14,15]

Superoxide and similar species of oxygen free radicals have also been implicated in carcinogenesis.[16] Mutagenesis through oxidative DNA damage is believed to be a frequent event in human cells. In particular, in rapidly proliferating cells of neonatal tissues, oxidative DNA damage could be exacerbated and may lead to mutations. A rapid increase in oxidative DNA lesions that is consistent with a sudden

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increase in partial oxygen pressure after birth was observed in rodent tissues, including liver, kidney and skin, suggesting that anti-oxidant defense systems were insufficiently developed in these tissues.[17] Oxygen free radicals were shown to play an important role in the development of hepatocellular carcinomas that were induced by oral contraceptives or hepatitis B virus infection.[18,19] There has been no report that suggests a crucial role of oxygen free radicals in hepatoblastoma tumorigenesis, but further work could focus on whether DNA damage generated by oxygen is causally associated with hepatoblastoma.

Prenatal corticosteroids, which enhance fetal lung maturation and prevent respiratory distress syndrome, were reported to stimulate anti-oxidant enzyme activity in lung tissue.[20,21] Whereas none of the cases in the present study was prenatally exposed to corticosteroids, 23% of controls had received prenatal administration of corticosteroids. Although this was not a statistically significant difference, it does suggest that prenatal steroid administration has an inhibiting effect on hepatoblastoma development. If a tumor arises as a consequence of oxygen DNA damage due to postnatal hyperoxia, prenatal steroid administration may prevent tumor development. There are conflicting reports regarding the effect of prenatal corticosteroids in stimulating anti-oxidant enzyme activity;[22] however, it is important to pursue the association between prenatal maternal corticosteroid treatment and lack of hepatoblastoma.

There have been few reports on the relationship between furosemide treatment and tumor development, except for one, which reported a mammary tumor occuring in mice receiving furosemide for a long period of time.[23] Furosemide is known to cause cholestasis by reducing bile salts without affecting bile flow.[24] Because long-term parenteral nutrition and resultant biliary cirrhosis was an underlying cause of hepatocellular carcinoma,[25] it is possible that furosemide-induced cholestasis initiates carcinogenesis in premature infants. However, there were only a small number of patients who had liver dysfunction or cholelithiasis and the occurence of cholestasis was infrequent in very low birthweight children with hepatoblastoma.[10] The results of the present study also indicate that these are not particular events in hepatoblastoma patients.

Although the results obtained in the present study suggest an association between postnatal intensive treatment and hepatoblastoma, the etiology of hepatoblastoma is still unclear. The possibility still remains that intensive treatment with oxygen and furosemide is a surrogate for some other causal factors. In addition, it has to be mentioned that the interpretation of the results must be made carefully because of the small number of cases included in the present study. Multinational collaborative studies will be necessary to explore the possibilities presented herein. In contrast, there is no doubt that further genetic and molecular studies should help us better understand the real reasons for this hepatic malignancy in low birthweight children. From the clinical point of view, because early detection is supposed to be related to resectable and curable disease, close monitoring of extremely premature infants, especially those who are treated intensively with oxygen and furosemide, is essential in follow up. In conclusion, extensive epidemiological and basic studies should be performed to determine the real reasons for the development of hepatoblastoma and to protect children of low birthweight from developing cancer.

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