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Neurotropic viruses and cerebral palsy: population based case-control study

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Abstract

Objective To investigate the association between cerebral palsy and direct evidence for perinatal exposure to neurotropic viruses.

Design Population based case-control study.

Setting Adelaide Women's and Children's Hospital Research Laboratory.

Participants and main outcome measures Newborn screening cards of 443 white case patients with cerebral palsy and 883 white controls were tested for viral nucleic acids from enteroviruses and herpes viruses by using polymerase chain reaction. Herpes group A viruses included herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus 8 (HHV-8); herpes group B viruses included varicella zoster virus (VZV) and human herpes viruses 6 and 7 (HHV-6 and HHV-7).

Results The prevalence of viral nucleic acids in the control population was high: 39.8% of controls tested positive, and the prevalence was highest in preterm babies. The detection of herpes group B viral nucleic acids increased the risk of developing cerebral palsy (odds ratio 1.68, 95% confidence interval 1.09 to 2.59).

Conclusions Perinatal exposure to neurotropic viruses is associated with preterm delivery and cerebral palsy.

Introduction

Intrauterine infection is postulated to be an important contributor to the development of cerebral palsy.¹⁻³ The herpes viruses (including cytomegalovirus (CMV), herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), and human herpes viruses 6, 7, and 8 (HHV-6, HHV-7, and HHV-8), and enteroviruses can all cross the placenta and infect the fetus. These viruses are potentially neurotropic. The likelihood of maternal infection resulting in infection of the fetus varies according to

the specific virus, whether the infection is primary or recurrent, and the gestational age of the fetus at the time of infection. Once the infection has crossed the placenta into the fetal circulation, the potential for neuronal damage is there, directly and also by the fetal inflammatory response.

Some viruses can persist for months or years after the initial infection. These viruses may have effects as long as 30 years after the original infection.

We investigated associations between potentially neurotropic viruses and cerebral palsy in a white Australian population. Our hypothesis was that evidence of perinatal viral infection may be associated with the development of cerebral palsy.

Methods

Selection of patients and controls

The study population comprised all children with cerebral palsy born in 1986-99 in South Australia to white mothers (n=443), ascertained by South Australia's cerebral palsy register. The controls were 883 babies born to white mothers from 1986 to 1999.⁴ We identified newborn screening cards for each case and control. We selected four potential controls from screening cards filed (by date of receipt) before (n=2) and after (n=2) the cards of cases. The date of birth of each control was within a few days of the case, the hospital from which we took the screening cards was of the same category (metropolitan teaching, metropolitan private, or country), and we took samples on roughly the same day of life as for the cases. The control population included a higher proportion of preterm infants than the general population, as many of the cases of cerebral palsy were born preterm and had been

P+ A table showing prevalences of neurotropic viruses in a population of newborns diagnosed with cerebral palsy is on bmj.com

ELPS This is the abridged version of an article that was posted on bmj.com on 6 January 2006: <http://bmj.com/cgi/doi/10.1136/bmj.38668.616806.3A>

Table 1 Odds ratios (with 95% confidence intervals) for specified viruses in babies with cerebral palsy at ≥ 37 weeks' gestation compared with controls at ≥ 37 weeks' gestation

Virus	All types of cerebral palsy (n=227)	Diplegia (n=49)	Hemiplegia (n=81)	Quadriplegia (n=65)
HSV (10)*	0.92 (0.44 to 1.92)	0.85 (0.20 to 3.67)	1.04 (0.35 to 3.03)	0.31 (0.04 to 2.33)
CMV (66)	1.30 (0.92 to 1.83)	1.03 (0.52 to 2.02)	1.04 (0.61 to 1.78)	1.62 (0.94 to 2.80)
Herpes group B (23)†	1.69 (0.98 to 2.92)	2.45 (1.02 to 5.89)‡	2.38 (1.15 to 4.92)‡	0.00 (0.00 to 0.89)‡
Herpes group A (73)§	1.25 (0.90 to 1.74)	0.95 (0.49 to 1.84)	1.11 (0.67 to 1.85)	1.45 (0.84 to 2.48)
Any herpes virus (88)¶	1.52 (1.09 to 2.13)‡	1.41 (0.74 to 2.67)	1.38 (0.83 to 2.30)	1.25 (0.71 to 2.20)
Enterovirus (10)	1.63 (0.74 to 3.62)	1.55 (0.35 to 6.90)	1.83 (0.60 to 5.58)	1.12 (0.25 to 4.97)
Any virus (96)**	1.64 (1.17 to 2.28)‡	1.58 (0.83 to 2.98)	1.43 (0.86 to 2.37)	1.29 (0.74 to 2.24)

Numbers of positive test results for each virus for cases of cerebral palsy are listed in parentheses in the first column.

*HSV-1, HSV-2, EBV, HHV-8. †VZV, HHV-6, HHV-7. ‡Significant at the 0.05 level. § HSV-1, HSV-2, EBV, CMV, HHV-8. ¶HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, HHV-8. **All herpes viruses or enterovirus.

referred to metropolitan teaching hospitals. Linkage, using South Australia's perinatal database, was successful for all cases and 1691 controls. We excluded 268/1691 (15.8%) controls because they had non-white mothers (n = 102), had a birth defect notified to the state's birth defects register (n = 161), or died in the first year of life (n = 37). Random numbers were then used to select controls from the remaining controls. We undertook all testing with blinding to case or control status.

Virus detection

The DNA viruses of interest included HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8. The RNA viruses included members of the Enterovirus family. We used two polymerase chain reactions to detect DNA viruses. We assigned results to the respective test groups: the first detected nucleic acids of HSV-1, HSV-2, EBV, CMV, and HHV-8, hereafter designated herpes group A, and the second detected nucleic acids of VZV, HHV-6, and HHV-7, hereafter designated herpes group B. We extracted punches of dried blood (1.2 mm) on newborn screening cards (collected by heel prick at 3-5 days of life) for DNA viruses. We chose a phenolic wash method to extract the newborn screening cards for RNA.

Sensitivity of virus detection

The minimum number of detectable viral nucleic acid copies was 2.8/bloodspot (5.6×10^3 /ml blood) for enterovirus, 1.6/bloodspot (3.2×10^3 /ml blood) for herpes group A viruses, and 15/microlitre (3.2×10^3 /ml blood) for herpes group B viruses.

Statistical analysis

We detected viral nucleic acids using newborn screening samples that had been stored for up to 18 years. We calculated prevalence proportions and confidence intervals of the viruses in the newborn screening cards tested.

As cases and controls had not been matched for covariates such as gestational age, we used all controls in our analysis. Data analysis considered cerebral palsy cases and controls by gestational age range (< 37 weeks, ≥ 37 weeks, and all gestational ages), type of cerebral palsy (diplegia, hemiplegia, quadriplegia, and all types) and virus.

Results

Results were obtained from a total of 414/443 cerebral palsy samples (93.5%) and 856/883 control samples (96.9%), a difference that may cause an underestima-

tion of the prevalence of viral exposure in the cerebral palsy cases (odds ratio 2.22, 95% confidence interval 1.26 to 3.93). Not all viruses gave a clear result for each card. The results are expressed as total readable results.

Prevalence of viruses in control population

In the control population, CMV was the most prevalent virus, with 228 babies (26.7%) testing positive. In addition, 42 babies (4.9%) were positive for more than one virus. Of the babies positive for multiple viruses, the most common combination observed was herpes group B and CMV, with a prevalence of 3.1% (1.9 to 4.6), followed by CMV and herpes group A, with a prevalence of 1.1% (0.5 to 2.1).

We investigated the effect of gestational age on the prevalence of these viruses because of the high rate of prematurity in the control population. CMV was significantly ($P < 0.01$) more prevalent in preterm (< 37 weeks) (82/247) than term (≥ 37 weeks) (146/608) babies (33.2% and 24.0%, respectively; odds ratio 1.57, 95% confidence interval 1.14 to 2.17). Herpes group A viruses were also significantly ($P < 0.05$) more prevalent in preterm (89/247) than term (167/608) babies (36.0% and 27.5%, respectively; 1.49, 1.09 to 2.04). The same trend was observed for the presence of any herpes virus, with 44.3% (98/221) of preterm babies testing positive for any herpes virus, compared with 35.7% (188/526) of term babies (1.43, 1.04 to 1.97).

Viruses and cerebral palsy

Of the 414 cases of cerebral palsy, 131 were diagnosed with diplegia, 119 with hemiplegia, 112 with quadriplegia, and 52 with other or unspecified subtypes.

All gestational ages

The primary analyses showed a significant association between any viral exposure and cerebral palsy at all gestational ages (1.30, 1.00 to 1.67). The detection of herpes group B viral nucleic acids increased the risk of developing all types of cerebral palsy, with an odds ratio of 1.68 (1.09 to 2.59). This increased risk was also observed for the diplegic (1.93, 1.03 to 3.61) and hemiplegic (2.07, 1.10 to 3.88) subtypes of cerebral palsy and herpes group B viruses. Associations did not reach significance for any of the other viruses.

Gestational age ≥ 37 weeks

The risk of developing cerebral palsy for a gestational age of 37 weeks or more increased with the detection of any herpes virus, and also with the detection of any virus (table 1). Herpes group B viruses were associated with the development of diplegia and hemiplegia.

Table 2 Odds ratios (with 95% confidence intervals) for specified viruses in babies with cerebral palsy at <37 weeks' gestation compared with controls at <37 weeks' gestation

Virus	All types of cerebral palsy (n=187)	Diplegia (n=82)	Hemiplegia (n=38)	Quadriplegia (n=47)
HSV (3)*	0.49 (0.13 to 1.87)	0.17 (0.00 to 1.75)	0.37 (0.00 to 3.85)	1.33 (0.27 to 6.46)
CMV (49)	0.72 (0.47 to 1.10)	0.88 (0.51 to 1.51)	0.72 (0.33 to 1.55)	0.61 (0.30 to 1.27)
Herpes group B (17)†	1.73 (0.83 to 3.59)	1.67 (0.65 to 4.30)	1.41 (0.38 to 5.17)	2.87 (1.09 to 7.59)‡
Herpes group A (50)§	0.65 (0.43 to 0.99)‡	0.78 (0.46 to 1.33)	0.63 (0.29 to 1.37)	0.54 (0.26 to 1.12)
Any herpes virus (61)¶	0.82 (0.54 to 1.25)	0.99 (0.57 to 1.71)	0.66 (0.30 to 1.43)	0.84 (0.42 to 1.66)
Enterovirus (3)	0.79 (0.19 to 3.35)	0.27 (0.01 to 4.89)	1.31 (0.15 to 11.52)	2.15 (0.40 to 11.44)
Any virus (64)**	0.88 (0.58 to 1.33)	0.97 (0.56 to 1.68)	0.74 (0.34 to 1.59)	1.01 (0.51 to 1.99)

Numbers of positive test results for each virus for CP cases are listed in parentheses in the first column.

*HSV-1, HSV-2, EBV, HHV-8. †VZV, HHV-6, HHV-7. ‡Significant at the 0.05 level. §HSV-1, HSV-2, EBV, CMV, HHV-8. ¶HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, HHV-8. **All herpes viruses or enterovirus.

Gestational age <37 weeks

The detection of herpes group A viral nucleic acids decreased the risk of developing all types of cerebral palsy for gestational age <37 weeks (table 2). Detection of herpes group B increased the risk for quadriplegia for gestational age <37 weeks.

Combination viruses at any gestational age

The presence of more than one virus was not associated with the risk of developing cerebral palsy at any gestational age (1.13, 0.65 to 1.98). Comparing term born babies with cerebral palsy with term born controls (1.29, 0.64 to 2.61), or preterm babies with cerebral palsy with preterm controls (0.98, 0.38 to 2.53), did not show any associations either.

Discussion

Perinatal exposure to and infection with viruses are associated with cerebral palsy in the newborn. Our findings reinforce the possibility of a complex and heterogeneous relation between perinatal exposure to neurotropic viruses and subtypes of cerebral palsy at different gestational ages. Other cofactors may trigger neurotropic damage. The findings were based on the detection of viral nucleic acids from newborn screening card blood spots collected within a few days of birth, and where detected, the findings indicate intrauterine or early neonatal viral exposure or infection.

Caveats

Post hoc analyses were performed on the individual viruses and their combinations, increasing the likelihood of identifying chance statistical associations. Because of the small numbers in some of the subanalyses, other associations cannot be excluded with confidence. Where associations are seen in one gestational age range, similar trends are seen in the same subgroup of cerebral palsy with the same virus in other gestational age ranges. This implies that these may indicate true causal relations and may not be attributed to chance. These associations require further study.

Viral associations with preterm birth and cerebral palsy

Exposure to viral nucleic acids seems very common in our control population, and the presence of intrauterine viral infectious agents was also associated with subsequent preterm delivery.

Our results showed a 1.5-2.5 times increased risk of developing cerebral palsy after perinatal exposure to

herpes group B viruses. The prevalence of herpes group B viruses was 7.6% for controls and 12.1% for babies with cerebral palsy, giving a potential attributable risk of 4.5% of all cases of cerebral palsy if a causal relation exists. These results also show an increased risk of developing cerebral palsy in term babies after perinatal exposure to any virus. In contrast, our results imply negative associations between perinatal exposure to viral nucleic acids and subsequent cerebral palsy for babies born before term. These conflicting results may be due to the higher prevalence of viral infection in control babies born before term, diluting any positive association between viral infection and cerebral palsy for preterm babies. Exposure late in gestation may have direct effects on the brain, whereas exposure early in gestation may result in preterm birth and increase the risk of neuropathology associated with prematurity.

Viral loads

Low viral loads may not be sufficient to precipitate preterm birth, but continuing intrauterine development in the presence of infection may allow the infection to contribute to permanent neuropathology. High viral loads may initiate or result in preterm birth and increase the risk of neurological damage associated with prematurity or infection, or both. We plan prospective studies quantifying viral loads. Prospective investigations are also required to follow women through pregnancy, testing antenatal maternal blood samples for viral nucleic acids, and umbilical cord blood to determine if there is active infection in the fetus or neonate. Widespread childhood vaccination against VZV, which has been available in South Australia for 10 years, will provide an opportunity to investigate the impact of maternal VZV infection and reactivation on the development of cerebral palsy.

Pathogenesis

It is unclear how perinatal exposure to viral infection causes subsequent brain damage and cerebral palsy. If the virus is able to cross the blood-brain barrier, it is capable of setting up infection in the brain and directly damaging vulnerable neuronal tissue. Alternatively, products of infection can be released locally and into the circulation, cross the blood-brain barrier and can cause direct damage to developing white matter.⁵⁻⁸

The high prevalence of exposure to viral infection in our control population indicates that "triggers" or cofactors including genetic susceptibility to infection and inherited thrombophilia are needed before brain damage can occur. An association between fetal

What is already known on this topic

Preterm birth and choriamnionitis are associated with cerebral palsy and infection with neurotropic viruses in neonates and infants can lead to neurological disability, including cerebral palsy

What this study adds

Stored dried neonatal blood spots, from babies who were diagnosed subsequently to have cerebral palsy, and from control babies, often contain nucleic acids from neurotropic viruses

Herpes group A (including cytomegalovirus) nucleic acids were found more often in neonatal blood spots of preterm control babies than in control babies born at term

Herpes group B nucleic acids were found more often in neonatal blood spots of babies who were diagnosed subsequently to have cerebral palsy than in control babies

The presence of neurotropic viral nucleic acids in the blood of newborns and the subsequent diagnosis of cerebral palsy are significantly associated

thrombophilia and cerebral palsy has been found in our cohort.⁹

Conclusions

Exposure to viral infection is common in newborn babies in South Australia, especially in preterm babies.

The risk of cerebral palsy is nearly doubled with exposure to herpes group B viruses but may require other factors or clinical events for brain damage and subsequent cerebral palsy to occur. Future studies are planned to investigate these factors.

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Lifetime effects, costs, and cost effectiveness of testing for human papillomavirus to manage low grade cytological abnormalities: modelling study

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Abstract

Objectives To predict the incremental lifetime effects, costs, and cost effectiveness of using human papillomavirus testing to triage women with borderline or mildly dyskaryotic cervical smear results for immediate colposcopy.

Design Modelling study.

Setting Three centres participating in NHS pilot studies, United Kingdom.

Population Women aged 25-64 with borderline or mildly dyskaryotic cervical smear results.

Interventions Screening using conventional cytology, liquid based cytology, and four strategies with different age cut-off points and follow-up times that used combined liquid based cytology and human papillomavirus testing (adjunctive human papillomavirus testing).

Results The model predicts that compared with using conventional cytology without testing for human papillomavirus, testing for the virus in conjunction with liquid based cytology for women (aged 35 or more) with borderline or mildly dyskaryotic cervical smear results would cost £3735 (€5528; \$6474) per life year saved. Extending adjunctive human papillomavirus testing in combination with liquid based cytology to include women aged between 25 and 34 costs an additional £4233 per life year saved. Human papillomavirus testing is likely to reduce



People involved in study, table, and figure are on bmj.com



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