Role of thrombophilic risk factors in children with non-stroke cerebral palsy

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Abstract

Background: Thrombophilic risk factors play an important role in the pathogenesis of perinatal stroke and resultant cerebral palsy (CP). The association between thrombophilia and CP caused by etiologies other than stroke is undetermined.

Methods: We assessed three genetic thrombophilic markers (mutation of Factor V Leiden [FV G1691A], 677T polymorphism of thermolabile methylenetetrahydrofolate reductase [MTHFR] and G20210A mutation of the prothrombin gene) in 49 pediatric patients with non-stroke CP and compared the findings with 118 apparently healthy controls. CP in the study group was due to periventricular leukomalacia (n=27), intraventricular hemorrhage (n=9), hypoxic ischemic encephalopathy (n=4), prematurity with no apparent complication (n=8) and intrauterine growth retardation (n=1). Twenty-five children had spastic diplegia, 20 had spastic quadriplegia and 4 had spastic hemiplegia. CP was graded as being severe in 26 children (53%).

Results: No significant difference in the prevalence of thrombophilic risk factors was found between the study and control groups. Twelve study children (24.5%) had at least one of the three thrombophilic mutations compared with 27 controls (23%).

Keywords: Thrombophilia; Cerebral palsy; MTHFR; Factor V leiden; Factor II
There was no significant difference in the prevalence of each thrombophilic risk factor in the various etiologic groups and in the subgroups of mild/severe CP and the control group.

Conclusion: These findings support the notion that thrombophilia neither contributes to the occurrence nor affects the clinical outcome and severity of non-stroke CP.

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Introduction

Cerebral palsy (CP) is a common disorder in childhood, occurring in 2–2.5 per 1000 live births [1]. Perinatal asphyxia and prematurity play an important role in the pathogenesis of CP, but its occurrence is often triggered by other predisposing factors, suggesting that more than a single risk factor may be involved [2,3]. Factors such as intrauterine exposure to infection, inflammation or coagulation disorders have been suggested as predisposing factors to brain injury [4–6]. The relation between CP and thrombophilia has not yet been defined [7]. It is well known that genetic prothrombotic risk factors play an important role in the pathogenesis of infantile thrombosis [8–10], childhood stroke [11–13], perinatal stroke [14,15] and resultant porencephaly [16] and hemiplegic CP [17,18]. The largest known contributor reported is Factor V Leiden mutation (FV G1691A) [15–19]. Whether or not thrombophilia plays a role in the pathogenesis of other brain lesions that result in CP has not been established. Some case series have demonstrated an increased risk of intraventricular hemorrhage (IVH), a recognized cause for CP, in preterm infants with thrombophilia [20,21]. Another population-based study reported contrary results, failing to demonstrate any increased risk of IVH and periventricular leukomalacia (PVL) in premature infants with thrombophilia [22]. Therefore, the contribution of thrombophilia to the development of non-stroke CP remains to be clarified.

Thrombophilia stems from acquired as well as inherited causes [23]. Several mutations in coagulation proteins are associated with an increased risk for thromboembolic complications. Resistance to activated protein C caused by an adenine 506 guanine (A506G) mutation in FV G1691A has been linked with an increased risk for venous thromboembolism [24] as well as childhood stroke [13]. Homozygosity for the cytosine 677 thymine (C677T) mutation in methylenetetrahydrofolate reductase (MTHFR) may slightly increase the risk for venous and arterial thrombosis [25], and the guanine 20210 adenine mutation in prothrombin (FII G20210A) has been associated with increased risk for venous thromboembolism and cerebral vein thrombosis [26].

The present study aimed to determine the prevalence of the abovementioned thrombophilic risk factors in non-stroke pediatric CP patients compared to those of a control group of apparently normal children.

Subjects and methods

Subjects

Between September 2001 and September 2003, we studied consecutive children with spastic CP who were referred to a large pediatric CP referral center in Israel. All children below 18 years of age who were diagnosed as having CP were considered suitable candidates for the study. Since the association of genetic thrombophilia and childhood stroke is well established, those children whose CP was caused by ischemic stroke were excluded. Children with a known familial thrombophilia, defined as either family history positive for early (<50 years old) thrombosis or presence of parental genetic thrombophilia, were excluded from our study as well. Diagnosis of non-stroke CP was made by absence of a past history of ischemic stroke and no evidence of porencephaly on an imaging study (computerized tomography [CT] or magnetic resonance imaging [MRI]). Forty-nine children (mean age 4.8±2.9 years) defined as non-stroke CP who met the inclusion criteria comprised the study group. All CP patients were diagnosed at 6–12 months (median 8 months). The children’s ethnic origin was classified as Ashkenazi Jewish, non-Ashkenazi Jewish, mixed Ashkenazi and non-Ashkenazi Jewish and Arabic (Table 1). The mean gestational age in the study group was 31.2±4.5 weeks and the mean birth weight was 1.65±0.87 kg.

The etiology for CP in the study group was PVL in 27 (55%), IVH in 9 (18%) and hypoxic ischemic encephalopathy (HIE) in 4 (8%); in eight children (16%) the etiology was defined as prematurity since no other cause for the development of CP could be traced. One child had severe intrauterine
growth retardation (IUGR). PVL was defined by one of two criteria: periventricular cystic lesions as seen on neonatal brain ultrasound (US) or brain MRI showing diminished periventricular white matter, irregular shape of the lateral ventricles or ventricular dilatation. IVH was defined according to the grading system of Volpe [27]. Forty-two (86%) children in our study group were preterm. In 7 children who were born at term, the etiology for CP was HIE (n=4), IVH (n=2) and IUGR (n=1). The study group included 25 children with spastic diplegia, 20 with spastic quadriplegia and 4 with spastic hemiplegia. The etiology in all children with hemiplegic CP was IVH.

The level of severity of CP was defined according to the functional ability and the type of CP. Specifically, severe CP was defined as either spastic quadriplegia and being either non-ambulatory or mentally retarded. The remaining CP children who were ambulatory without mental retardation were defined as having mild CP.

The control group consisted of 118 apparently healthy children who were recruited prior to undergoing an elective surgical procedure or routinely visiting ambulatory childcare clinics: none had known familial thrombophilia defined as either family history positive for early (<50 years old) thrombosis or presence of parental genetic thrombophilia, history of previous thromboembolic events or neurological abnormalities. The distribution of gender and ethnic origin was similar in both groups (Table 1). The mean age of the control group was 8.2±3.8 years (Table 1). The difference in age between the two groups had no impact upon our results since the studied inherited mutations are genetically transmitted and not age-dependent.

Methods

We evaluated three thrombophilic markers: mutation of Factor V Leiden, C677T polymorphism of thermolabile MTHFR and G20210A mutation of the prothrombin gene. Citrated blood (0.38%) was drawn for DNA analysis of the thrombophilic mutations from all the children at the time of study enrollment. Molecular diagnosis of the FV G1691A mutation was performed as described by Dalback [28]. The mutation in the MTHFR gene was tested by the method of Frost et al. [25]. The mutation in the prothrombin gene was detected by means of a slight modification of the method of Poort et al. [26] as described by Salomon et al. [29].

A questionnaire on birth weight, perinatal history, family history, type and severity of the CP, motor function, and cognitive ability was filled out for all the children in the study group. The information was obtained from the medical charts and the children’s parents. The study was approved by the Ethics Committee of the Tel Aviv Sourasky Medical Center.

Statistical analysis

The unpaired t-test was used for between-group comparison of birth weight and age. Prevalence of prothrombotic risk factors in the patients and control subjects was calculated by Fisher’s exact test. The significance level was set at 0.05. The odds ratios and 95% confidence intervals were calculated in the study group compared with controls, and in each of the three etiologic groups of CP compared with controls. The same analysis was performed to compare the mild with severe CP patients.

Results

Prothrombotic risk factors in the study and control groups

Overall, 12 of the 49 children with non-stroke CP (24.5%) had at least one of the three thrombophilic mutations compared with 27 of the 118 normal controls (22.9%). Four study group children had FV G1691A, 9 had an MTHFR mutation and one had FII G20210A mutation. Two children had combined thrombophilic mutations: one FV G1691A and MTHFR and one FII G20210A and MTHFR. Both were considered only once for the statistical analysis. No children in the control group had combinations of thrombophilia risk factors. There was no significant difference in the prevalence of each thrombophilic risk factor among the study patients and the normal controls (Table 2).
Prothrombotic risk factors in the various etiologic groups

There was no significant difference in the prevalence of each thrombophilic risk factor in the various etiologic groups. Seven children in the PVL group (26%) had at least one of the three thrombophilic mutations, 2 (22%) in the IVH group, 1 (25%) in the HIE group and 2 (25%) in the prematurity group.

Mild vs. severe CP

Twenty-six of the CP children (53%) were defined as having severe CP and the remaining 23 children (47%) as having mild CP. Seven of 26 children (27%) with severe CP were found to have at least one prothrombotic risk factor compared with 5 children (22%) in the mild CP group. There was no significant difference in the prevalence of each thrombophilic risk factor among the mild and severe CP groups (odds ratio, 1.32; 95% CI, 0.51–7.82). Among the children with severe CP, 3 had FV G1691A and 4 had an MTHFR mutation. Among the children with mild CP, 1 had FV G1691A, 5 had an MTHFR mutation and 1 had a FII G20210A mutation (two had combined mutations).

Table 2  Thrombophilic risk factors in the study versus the control groups

<table>
<thead>
<tr>
<th>Children with non-stroke cerebral palsy (n=49)</th>
<th>Controls (n=118)</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombophilia</td>
<td>12a (24.5%)</td>
<td>27 (22.9%)</td>
<td>1.09</td>
</tr>
<tr>
<td>FV G1691A</td>
<td>4 (8.2%)</td>
<td>5 (4.2%)</td>
<td>2.00</td>
</tr>
<tr>
<td>MTHFR T677T</td>
<td>9 (18.4%)</td>
<td>19 (16%)</td>
<td>1.17</td>
</tr>
<tr>
<td>FII G20210A</td>
<td>1 (2%)</td>
<td>3 (2.5%)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

a Two children in the CP group had combined risk factors.

In our study the prevalence of thrombophilia markers was similar to that of the controls in each etiologic subgroup of CP. The majority of our CP patients were born prematurely (n=42) and suffered from complications of prematurity, e.g. PVL and IVH. Therefore, we conclude that thrombophilia did not contribute to the occurrence of CP in premature infants nor in those with a history of PVL or IVH. The fact that the majority of the study group were premature should not affect the results since the studied inherited mutations are genetically transmitted and do not depend on gestational age. Moreover, previous study demonstrated that the prevalence of thrombophilia in preterm Israeli newborns was similar to the healthy pediatric Israeli population [17]. However, we cannot rule out the impact of possible undiagnosed maternal thrombophilia upon the occurrence of prematurity in our study population.

The prevalence of thrombophilic markers did not differ among patients with mild or severe CP. Therefore, the presence of thrombophilic markers had no impact upon the neurological outcome of our patients. On the contrary two patients with combined thrombophilic mutation had mild CP.

In summary, the results of the current study support the notion that thrombophilia neither contributes to the occurrence nor affects the clinical outcome of CP that is not attributed to stroke, in children.

Acknowledgment

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References


