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Serum Immunoreactivity to S-100 in Children with Cerebral Palsy and Delayed Development and in Their Healthy Parents

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Abstract

The passive immunization of pregnant female rats to S-100 protein often leads to ultra-structural abnormalities in the brain glial structures of the offspring of these rats and induces signs of delayed development in the fetal brain. Additionally passive immunization of pregnant animals with certain antigens induces permanent Ag-specific changes in the immune response of their offspring. The purpose of this study was to investigate serum immunoreactiviy (SIR) to S-100 in cerebral-palsied and developmentally-delayed children as well as in their healthy parents and to evaluate its significance related to radiologic findings of brain MRI and single photon emission computed tomography (SPECT). The subjects were children with cerebral palsy and delayed development that had abnormal findings on brain MRI or Brain SPECT. SIR to S-100 protein was measured by ELISA method in the patients, their healthy parents, 20 normal adult controls and 22 normally developed children. The SIR to S-100 protein was significantly higher in the cerebral-palsied and developmentally-delayed children when compared to that of the normal control group children. Increased SIRs were detected in healthy mothers but not in their fathers. There was no difference of SIR between the cerebral-palsied and developmentally-delayed children or any significant difference of SIRs according to the findings of the brain MRI or to developmental quotients. But, the SIRs to S-100 protein were higher in the group of more abnormal findings on brain SPECT.

Key Words: S-100 protein, cerebral palsy, delayed development

INTRODUCTION

The S-100 protein is a neuron-specific protein which is synthesized in astroglial cells in all parts of the central nervous system.[1] The passive immunization of pregnant female rats to S-100 protein leads to ultra-structural abnormalities in the brain glial structures of the offspring of these rats and this could be considered as one of the mechanisms in developmental brain dysfunction.[2] Poletaev reported that serum immunoreactivity to S-100 protein in children with cerebral palsy and mental retardation and their healthy mothers was higher than that of normal control children or normal control adults.[1] He suggested that vertical epigenetic transfer of the humoral immune profile to S-100 protein plays an important role in the inborn malformation of the human nervous system.

High levels of S-100 protein in cerebrospinal fluid have been demonstrated in patients with various neurological diseases and injuries.[3,4] The study of Takayasu et al. have indicated that there is a quantitative relation between the degree of cell damage in the central nervous system and the concentration of S-100 proteins in CSF in patients with neurologic lesions.[5] This protein is not detectable in serum under normal conditions.[6] Following glial tissue damage with subsequent disruption of the blood-brain barrier, this protein is released into the serum. Recently, one study reported that serum measurements of S-100 protein may be useful tool for the detection of minor head injury.[7]

This study was designed to investigate the serum immunoreactivity to S-100 protein in the children with cerebral palsy and delayed development resulting from central nervous system dysfunction. Additionally,

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it was to evaluate the relationship between SIR to S-100 protein and the radiologic findings of brain MRI, SPECT and the developmental status of these children.

MATERIALS AND METHODS

Children with motor developmental problems who displayed abnormal findings on Brain MRI or Brain SPECT study were included as subjects. Children who were unable to walk independently until 15 months old (corrected age), without any abnormalities in the movement pattern or posture were grouped into the delayed development group. Children who had experienced movement or postural abnormalities due to non-progressive lesion of an immature brain were grouped into cerebral palsy group.

The patients underwent electrodiagnostic evaluation and muscle enzyme tests to rule out combined peripehral nerve and muscle abnormalities. Seventeen boys and 23 girls participated in this study. Their mean age of the children was 18.1 (SD=15.6) months old of age for boys and 18.6 (SD=11.5) months old of age for girls. A brain MRI was performed using a 1.5 Tesla Magnetom (General Electric Medical Systems, Milwaukee, Wis, USA). The T1-weighted images were obtained in an axial orientation and the T2-weighted images were obtained in axial and coronal orientations. Brain SPECT following intravenous injection of ^{99m}Tc-ECD was performed. All children were sedated with Sephamin 0.5 ml/kg for intravenous injection and chloral hydrate 0.1 g/kg for enema application. Developmental assessments in the children were performed using Mnchner Functionelle Entwicklungs Diagnostik (MFED) test, which is a tool for assessing development in children. The MFED test is a reliable and objective developmental test for children.[8] The developmental quotient was calculated as the mean developmental age of the MFED category divided by chronological age.

Blood samples of both patients and their healthy parents were obtained for checking S-100 immunoreactivity in serum. Additionally, blood samples of 22 normally developed children without any neurological abnormalities whose mean age was 23.9±15.2 months and the serum of 20 healthy adults were collected as controls.

Highly pure S-100 antigen was purchased from Fitzgerald (Fitzgerald Industrial International Inc. Concord, MA, USA). The S-100 antigen was diluted with 1 ml distilled water (1 mg/ml) and a working solution of 1 : 200 was used in a sodium carbonate buffer (0.05 mol/L NaHCO3/Na2CO3, pH 9.6). We added 100 uL (0.5 ug/well) of S-100 antigen to a 96-well microtiter plate (Nunc Co, Coppenhagen, Denmark) and incubated overnight at 4°C. Following incubation and washing three times with PBS containing 0.05% Tween (PBS/TW), we blocked the plate with 200 uL of PBS/TW containing 1% bovine serum albumin (PBS/TW/A) for 1 hour at room temperature. After blocking, the solution in each well was thrown away. The serum of the subjects was diluted 1 : 2 in PBS. One hundred uL of diluted serum was added to each well and incubated for 1 hour at 37°C. After incubating the serum in microtiter wells, we washed the microtiter plates three times with PBS/TW and added 100 uL of anti-human IgG, tagged with peroxidase and diluted 1 : 50 in PBS/TW/A, to each well (Behring, Diagnostic GmbH, Marburg, Germany). After incubating for 1 hour at 37°C, we washed the microtiter plates three times with PBS/TW and added 100 uL of tetramethyl benzidine dihydrochloride (TMB), and then incubated it for 30 min at room temperature for color development. We added 0.N sulfuric acid to stop the reaction and read the optical density at 450 nm. All tests were done in duplicate. The serum immunoreactivity (SIR) was calculated as a percentage by comparing it to the mean serum anti S-100 reactivity levels of the control subjects. A SIR of more than 2.0 standard deviation above the mean value of the value for normal controls was considered an abnormal value.

The mean values and standard deviation (SD) were also calculated. Data was analyzed using student *t* test or multiple comparison (Tukey's Studentized Range) test after one-way ANOVA. A statistically significant difference was accepted as $p<0.05$.

RESULTS

The mean of serum immunoreactivities (SIRs) in the patients with cerebral palsy was 349.68%, which was significantly higher than that of the healthy control children. The mean of SIRs in the patients with delayed development was 317.08%, which was also significantly higher than that of the healthy control children. But there was no difference of SIRs observed

between the cerebral palsy and delayed development groups (Table 1). The mean of the SIRs to S-100 in the healthy mothers of patients was significantly higher than that of the adult control group. However, the mean of SIRs to S-100 in the healthy fathers of patients was not significantly different with that of the adult control group (Table 2).

The mean SIRs of children with normal brain MRI findings was 335.49% and the mean SIRs of children with abnormal brain MRI findings was 336.11%. A comparison of SIRs between these two groups according to brain MRI findings showed no significant difference (Table 3). In our previoius our study[9] of 60 cerebral palsied children, abnormal hypoperfusion on the thalamus on the brain SPECT was seen in 96.7% of cases and abnormal hypoperfusion on the cerebellum in 45.0%. In this study, the abnormal hypoperfusion on the thalamus or cerebellum was a basic abnormal finding in the patients and 14 children displayed additional abnormal hypoperfusion on other brain areas. Therefore, abnormal findngs on brain SPECT were grouped into two groups in this study. Group 1 was the group that showed decreased perfusion on either the cerebellum or thalamus on brain SPECT and group 2 was group that showed decreased perfusion on cerebellum, thalamus or any other area. The mean SIRs of group 2 on the brain SPECT was 430.01% and mean SIRs of group 1 on brain SPECT was 289.93%. The mean SIRs of the children who had more abnormal brain SPECT findings was significantly higher than that of the children with fewer abnormalities on the brain SPECT (Table 4).

Nine patients had normal values of SIRs and 31 patients had abnormally high SIRs. The developmental quotient between the children with normal SIRs and abnormal SIRs was not significantly different (Table 5). SIRs to S-100 were elevated in 35 of 40 patients (87.5%). Blood samples were obtained from 39 of their healthy mothers and 33 their healthy fathers. Twenty-four (61.5%) of 39 healthy mothers had abnormally elevated SIRs to S-100. Concordant hyperreactivities with S-100 protein in mother-child pairs were detected in 23 of 39 cases (59.0%) of the

Table 1. Serum Immunoreactivity (SIR) to S-100 in Patients and Healthy Children

Diagnosis	SIR (%)
Cerebral palsy (n=23)	349.68±183.50*
Delayed development (n=17)	317.08±131.29*
Healthy children (n=22)	100.00±34.93

* $p<0.05$ vs healthy children.
Multiple comparison after one-way ANOVA.

Table 2. SIR to S-100 in Parents & Adults Control

Diagnosis	SIR (%)
Mother (n=39)	252.61±138.92*
Father (n=33)	130.59±90.06
Adult control (n=16)	100.00±49.62

* $p<0.05$ vs adult control by student t test.

Table 3. MRI findings and SIR to S-100

MRI findings	SIR (%)
Normal (n=18)	335.49±146.50
Abnormal (n=22)	336.11±177.57

$p>0.05$.

Table 4. SPECT Findings and SIR to S-100

SPECT findings	SIR (%)
Group 1 (n=26)	289.93±127.71*
Group 2 (n=14)	430.01±181.75*

Group 1: decreased perfusion on cerebellum or thalamus.
Group 2: decreased perfusion on cerebellum, thalamus or any other area.
* $p<0.05$.

Table 5. Developmental Quotient (DQ) According to SIR Normality

Developmental Area on MFED*	DQ (%)	
	Normal SIR (n=9)	Abnormal SIR (n=31)
Gross motor	62.61±27.51	52.45±27.19
Fine motor	68.42±31.59	65.15±23.81
Perception	81.02±36.70	73.18±31.39
Speech	76.83±28.95	69.67±29.78
Sociality	82.49±36.91	73.18±30.16
Independence	2.38±23.30	63.82±21.79

* Mnchner Functionelle Entwicklungs Diagnostik.

children with cerebral palsy and delayed development.

DISCUSSION

The S-100 protein is an acidic calcium-binding protein (molecular weight, 21000) constituting a major component of the cytosol found predominantly in astroglial cells[10,11] and normally extremely-weak immunogens. The S-100 protein can often be considered as an antigen-target in different neuropsychopathologic diseases such as schizophrenia, cerebral atrophy, depression, dementia and mental retardation.[12] The passive immunization of pregnant female rats to S-100 protein often leads to ultrastructural abnormalities in the brain glial structures of the offspring of these rats and induces signs of delayed development in the fetal brain.[13] This means that vertical epigenetic transfer of immune profile may be one of the causes of developmental brain dysfunction. The results we obtained also showed elevated immunoreactivity to S-100 protein in many the patients with cerebral-palsy and as well as developmentally-delayed children. That result was very similar to the report of Poletaev et al.'s.[1] However, in the healthy mothers of patients, elevated SIRs were detected in 61.5% and concordant hyperreactivity to S-100 protein in mother-child pairs was detected in 59.0%. Although the concordant hyperreactivity to S-100 protein is much lower than the 80% reported by Poletaev et al.,[1] that percentage is quite surprisingly high as one of the causes of cerebral palsy. There are so many diseases and conditions that can injure the developing brain and lead to cerebral palsy. Additionally approximately one quarter of all cases of cerebral palsy still have no definable cause.[14] More recently, problems during intrauterine development have been considered as a major cause of cerebral palsy.[15] The relatively high percentage of concordant hyperreactivity of mother-child pairs in this study suggests that vertical epigenetic transfer of the immune profile to S-100 protein might be considered as one of the causes resulting in cerebral palsy and delayed development. In this study, blood samples of the sibling of patients were not obtained. If the SIRs to S-100 protein in siblings had been compared to that of the patients, it would have been very helpful to get certain conclusion.

Cerebral palsy is a posture and movement disorder resulting from immature brain insult. Its clinical findings are very heterogeneous and the functional outcomes are quite variable. There have been many trials to predict clinical outcome in children with cerebral palsy. The radiologic tests of brain MRI and brain SPECT are thought to be helpful in predicting the clinical outcome in these patients.[16-20] However, there are still discrepancies between the findings of the radiologic study and the clinical outcome in some cases. Therefore, if a marker that can quantify brain injury could be determined, treatment planing and predicting outcomes in cerebral palsied patients would be much easier.

The concentration of S-100 in CSF was considered as a sensitive marker of brain damage after head trauma, cerebral hypoxia, cerebral bleeding and ischemic stroke.[21] A recent study has shown that S-100 in serum can be used as a peripheral marker of ischemic focal brain damage.[22] There was also a quantitative relation between the degree of cell damage in the brain and the S-100 concentration in CSF in patients with neurological lesions.[23,24] In our study, the serum concentration of S-100 was indirectly measured by serum immunoreactivity to S-100 protein. The results of this study did not show that there was difference in SIRs between children with abnormal brain MRI and those with normal brain MRI finding. Additionally, no significant difference in developmental status was observed between children with normal SIRs and abnormal SIRs. However, there was a significant difference of SIRs to S-100 protein according to the findings of brain SPECT. These results did not reveal a definite relation of SIRs to S-100 protein to the severity of brain injury. But brain SPECT has been considered a valuable tool for thorough neurologic assessment in cerebral palsy and as having a potential to predict the outcome of mental development and limb involvement.[19,20] From that point of view, the difference of immunoreactivity between the two groups according to brain SPECT may be considered as positive evidence of a useful marker for brain injury.

There were some limitations in this study. First, the number of subjects was not sufficient. Second, the clinical features of children represented a broad spectrum, from motor developmental retardation to severe spastic quadriplegia. Third, development in children is influenced by many other environmental factors such as socioeconomic status or nutritional status as well as neurophysiologic factors,[25] and these factors affecting development were not controlled for in this

study. Therefore, if these limitations were controlled for in further study, the role of SIRs to S-100 protein in relation to quantified brain injury may be delineated more clearly.

In conclusion, hyperproduction of supposedly pathologic antibodies against brain specific S-100 antigen was demonstrated in cerebral-palsied and developmentally-retarded children and their healthy mothers, but not in their fathers. This result supports the hypothesis that maternal connected pathological changes in the immune profile of the child to S-100 protein may be one cause resulting in disturbances in the developing nervous system of the immature brain. From the perspective of the usefulness of SIRs to S-100 protein as an adjuvant method for quantifying measurement of brain injury, our study did not show any conclusive evidence.

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