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ORIGINAL ARTICLE

Changes of Nerve Growth Factor in Amniotic Fluid and Correlation with Ventriculomegaly[△]

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Key words: nerve growth factor; amniotic fluid; fetal ventriculomegaly

Objective To detect the change of nerve growth factor (NGF) level in human amniotic fluid during gestation, and to explore the relationship between this change and fetal ventriculomegaly (VM).

Methods The studied subjects (collected from 2004 to 2007) were divided into four groups, including the second-trimester pregnancy group ($n=113$), third-trimester pregnancy group ($n=110$), fetal cerebral VM group ($n=12$), and healthy control group ($n=12$) which matched with the VM group in gestational weeks. The amniotic fluid specimens were obtained during amniocentesis or cesarean section. The NGF levels in amniotic fluid were detected with enzyme-linked immunosorbent assay.

Results A significantly negative correlation was found between gestational age and the NGF level in amniotic fluid ($r=-0.6149$, $P<0.0001$). The NGF level in patients with fetal VM was significantly lower than that in healthy controls (33.95 ± 29.24 pg/mL vs. 64.73 ± 16.21 pg/mL, $P=0.024$).

Conclusion NGF levels in amniotic fluid may be a sensitive marker for fetal VM.

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NEUROTROPHINS (NTs) constitute a family of structurally related molecules, including nerve growth factor (NGF), brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, NT-3, and NT-4/5.¹ NTs support the survival and maintenance of specific populations of neurons both in

peripheral and central nervous system.² As a member of this family, NGF is an important neurotrophic factor in cerebral cortical development for its ability to stimulate neuronal precursor cell proliferation. NGF plays a role in promoting the growth and differentiation of neurons, regulating the survival of motor neurons, exciting and stimulating the growth of axons and dendrites of neurons, and inducing the destined growth of neuronal fiber.³

Ventriculomegaly (VM) is defined as the presence of an excess of fluid in the lateral ventricles of the developing brain.⁴ Fetal VM is one of the most common findings in ultrasound screening for fetal malformation during preg-

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nancy, with an incidence of 1-2/1000.^{5,6} The diagnosis is usually made when the diameter of one or both ventricles is ≥ 10 mm from 14 weeks of gestational age to term. VM is severe when the width of the ventricular is over 15 mm.⁷ However, the sensitivity of ultrasound is low when the gestational age is less than 24 weeks.⁷ Moreover, VM is among the most common false positive diagnoses at ultrasound screening for fetal malformation.⁸ A novel sensitive approach is therefore necessary for timely detection of VM. In this study, we detected the change of NGF in amniotic fluid during pregnancy, and explored the correlation of NGF in amniotic fluid with fetal VM.

PATIENTS AND METHODS

Experimental model

The subjects (collected in the period of 2004-2007) were divided into four groups. The first was second-trimester pregnancy group ($n=113$). The second was third-trimester pregnancy group ($n=110$). The third group was fetal VM or choroid plexus cyst (CPS) ($n=12$), detected by ultrasound performed at 20-24 weeks. The subjects in the forth group, healthy controls ($n=12$), were detected with neither VM nor other abnormalities in ultrasonography, and matched with VM group in gestational age. All the general clinical data were recorded including maternal age, gestational weeks, complications, and abnormal history of pregnancy.

Sample collection

In the second-trimester pregnancy group, amniotic fluid was obtained during amniocentesis. Indications for amniocentesis included advanced maternal age, high risk result of triple screen test, and abnormal history of pregnancy. The amniotic fluid in the third-trimester pregnancy group was collected during cesarean section, including one case of twin gestation with two amniotic fluid samples and one triplet with three amniotic fluid samples. In VM group, the amniotic fluid was collected during amniocentesis for chromosomal karyotype or pregnant termination. In control group, the amniotic fluid was obtained during amniocentesis for advanced maternal age. Each sample was sterilized and collected into two cups in order to duplicate the experiment. The samples were centrifuged for 10 minutes at 3000 rpm (relative centrifugal force $10 \times g$) within 4-6 hours after collection, and frozen at -80°C until assayed.

Enzyme-linked immunosorbent assay (ELISA)

Two-site ELISA was performed according to the manufacturer's instructions (Promega ImmunoAssay System,

Beijing, China). Briefly, 100 μL of coating buffer containing the capture antibody (anti-NGF polyclonal antibody) was added to 96-well ELISA plates and incubated for 14-18 hours at 4°C . The plates were then washed and blocked overnight at 4°C with blocking buffer. Subsequently, 100 μL NGF at different concentrations (original solution and serial dilutions gradually diluted in the ratio of 1 : 2, until reaching the ratio of 1 : 2000) and samples were added to the wells, and the plates were incubated at room temperature for 6 hours with shaking. Then the wells were incubated with anti-NGF monoclonal antibody (Promega, Madison, WI, USA) at 4°C for 16-20 hours. After washing, 100 μL detection antibody (anti-rat IgG-horseradish peroxidase conjugate, Promega) was added into the plates and incubated at room temperature for 2.5 hours with shaking. Following multiple washing, the wells were treated with 100 μL of tetramethylbenzidine solution and incubated in the dark at room temperature for 5-10 minutes. Development was stopped by adding 50 μL of 1N hydrochloric acid. The plates were read at OD 450 nm.⁹

Statistical analysis

Statistical analysis was performed using SAS V 9.1 software. The data are expressed as means $\pm$ SD. Normal distribution of the data was tested. Inter-group differences were tested with the Kruskal-Wallis analysis of variance (ANOVA). An analysis of covariance was performed between the VM group and control group in order to rule out the impact of gestational ages. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the four groups were described in Table 1. Twelve cases with VM or CPS were determined by ultrasonography (Table 2).

According to the results in second-trimester pregnancy group and third-trimester pregnancy group, the NGF levels in amniotic fluid were found decreased along with the increase of gestational age (Fig. 1). There was a strong negative correlation between the NGF levels and gestational age ($r = -0.6149$, $P < 0.0001$). The NGF level in amniotic fluid in fetal VM group was significantly lower than that in healthy controls (33.95 ± 29.24 pg/mL vs. 64.73 ± 16.21 pg/mL, $P = 0.024$, Table 3). The NGF level in amniotic fluid was 56.60 ± 26.73 pg/mL in second-trimester pregnancy group, and 12.62 ± 13.95 pg/mL in third-trimester pregnancy group, significantly higher in the former group ($P < 0.0001$, Table 3).

Table 1. Demographic and clinical information of the four studied groups[§]

Groups	Maternal age (yr)	Gestational age (week)
Second-trimester pregnancy (n=113)	33.8±5.3	20.6±1.2
Third-trimester pregnancy (n=110)	30.2±4.5	38.6±1.4
Ventriculomegaly (n=12)	29.7±4.1	24.4±4.7
Healthy control (n=12)	30.1±4.3	21.8±0.8

§: Plus-minus values are means±SD.

Table 2. Ultrasonography description of fetal nerve system abnormalities in VM group (n=12)

Case	Maternal age (yr)	Gestational week	NGF level (pg/mL)	Ultrasonography finding	Complications
1	36	19	28.33	CPS	No
2	28	26	7.31	CPS	Strawberry-shape craniocerebral malformation
3	33	33	31.92	VM	No
4	29	31	21.54	VM	Holoprosencephaly
5	29	24	17.55	Mono-atria	Abnormalities in limbs
6	23	22	35.37	CPS	No
7	25	28	32.84	VM	No
8	34	34	28.46	VM	Cyst in cerebellum
9	26	22	24.20	VM	No
10	32	20	12.37	VM	No
11	35	26	120.34	VM	No
12	27	21	47.21	CPS	No

VM, ventriculomegaly; CPS, choroid plexus cyst; NGF, nerve growth factor.

Table 3. Comparison of NGF level between ventriculomegaly group and healthy controls; comparison of second-trimester group with third-trimester group using analysis of covariance[§]

Groups	NGF level (pg/mL)
Second-trimester group (n=113)	56.60±26.73 [†]
Third-trimester group (n=110)	12.62±13.95
Healthy control group (n=12)	64.73±16.21
VM group (n=12)	33.95±29.24*

§: Plus-minus values are means±SD.

*P=0.024 compare with healthy control group; [†]P<0.0001 compare with third-trimester group.

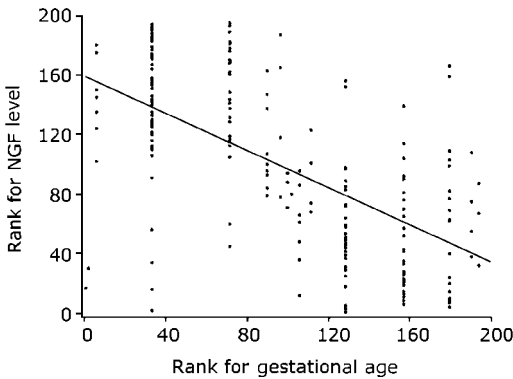


Figure 1. Relationship between the NGF level in amniotic fluid and gestational age in second-trimester pregnancy group (n=113) and third-trimester pregnancy group (n=110). The scatter diagram shows a significantly negative correlation between NGF level and gestational age ($r=-0.6149$, $P<0.0001$).

DISCUSSION

In this study, we first found that NGF level decreased from second-trimester to third-trimester, indicating that NGF level in human amniotic fluid declines as gestational age increases. This change of NGF in amniotic fluid corresponds with the development of fetal nerve system.¹⁰ A study by Mashayekhi et al shown that the total NGF level in cerebrospinal fluid drops in the process of chick embryo development.¹¹ These findings suggest that NGF levels might increase in the early stage of embryonic development, and reach a peak at a specific time, then decrease while development proceeds. Further studies in human are necessary to confirm that hypothesis.

Hydrocephaly means VM with increased cerebrospinal fluid pressure and usually an abnormally large head circumference. VM can result from different conditions, such as abnormal turnover of cerebrospinal fluid, abnormal migration and proliferation of neurons, agenesis of the corpus callosum, and holoprosencephaly.¹² Since it is impossible to measure the cerebrospinal fluid pressure of fetus in uterus, the finding of VM in prenatal ultrasound screening is relied upon to indicate abnormal accumulation of cerebrospinal fluid in cerebral ventricles.⁴ However, the sensitivity is low at gestational ages shorter than 24 weeks, with only 57% of severe VM cases diagnosed before 24 weeks.⁷ The low sensitivity before 24 weeks probably re-

flects the natural history of the disease, not a measurement error. Because cerebrospinal fluid accumulation in the ventricular system develops over time and VM becomes evident only later in gestation.⁴ In order to rule out the influence of gestational weeks, we selected the healthy control group matched with VM group in gestational age. Inter-group comparison revealed significantly lower NGF level in VM group than that in healthy control group. A negative correlation between NGF levels in amniotic fluid and fetal VM was found, consistent with the finding by Marx et al.¹² Although the relationship between NGF level in amniotic fluid and the development of fetal nerve system has not been clarified yet, decreased NGF levels in amniotic fluid may be a marker for the presence of fetal VM and other central nervous system abnormalities.¹²

As mentioned above, fetal VM was mainly detected with ultrasound screening and magnetic resonance imaging for fetal malformation. However, VM is among the most common false positive diagnoses at ultrasound screening for fetal malformation.⁸ Our findings suggest that the NGF level in amniotic fluid may help in diagnosing VM prenatally with high accuracy. However, our sample size was not large enough. Besides, amniocentesis in second-trimester pregnancy may have the risk of fetal loss at the incidence of about 0.5%.¹³ Further research is required to confirm the finding of the present study, and to identify the change of NGF level in blood during pregnancy and its correlation with the NGF level in amniotic fluid. We hope a safer method could be developed in earlier detecting fetal VM and other cerebral abnormalities.

In conclusion, our study showed that during pregnancy, the NGF level in amniotic fluid decreases while gestation proceeds. The NGF level is significantly lower in fetal VM than in healthy controls. A significantly negative correlation has been found between the NGF level and fetal VM.

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