The role of amino acids in spina bifida

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Summary

Our objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with spina bifida in the second trimester of pregnancy. Fifteen pregnant women who had fetuses with spina bifida detected by ultrasonography (spina bifida group) in the second trimester and 19 women who had abnormal triple screenings indicating an increased risk for Down's syndrome but had healthy fetuses (control group) were enrolled in the study. Amniotic fluid was obtained by amniocentesis. The chromosomal analysis of the study and control groups was normal. Levels of free amino acids were measured in amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer). The mean levels of alanine, cystathionine, cysteine, phenylalanine, tryptophane, and tyrosine amino acids were found to be significantly higher in fetuses of the control group than in the spina bifida group ($p < 0.05$). The detection of significantly higher amino acid concentrations in the amniotic fluid of healthy fetuses suggests loss of amino acids from the fetus through the spinal cord may contribute to the etiology of spina bifida.

Key words: Spina bifida; Amino acids; Amniocentesis.

Introduction

Spina bifida is a congenital disorder caused by the incomplete closing of the embryonic neural tube. Vertebra overlying the spinal cord are not fully formed and remain unfused and open. There may be a fluid-filled sac surrounding the spinal cord in amniotic fluid of pregnant women. Spina bifida occulta, myelomeningocele, meningocele and lipomeningocele are all types of neural tube defects. The incidence of spina bifida occulta is approximately 10% of the population [1].

The cause of spina bifida is not known in most cases and the etiology remains complex and poorly understood. It is generally agreed that most cases are of multifactor origin. Environmental factors are also important. Maternal age, alcohol consumption, maternal exposure to excess vitamin A, and folic acid deficency may be associated with the pathogenesis of neural tube defects [2].

The aim of the study was to determine the amino acid concentrations of spina bifida cases in amniotic fluid in the second trimester of pregnancy. We hypothesized that concentrations of amino acids may be a cause or the result of spina bifida cases.

Material and Methods

The study was performed at the Prenatal Diagnosis Unit of our Research Hospital between January 2009 and June 2011 and was approved by the Institutional Review Board and Ethics Committee of the university hospital. Written informed consent was obtained from all participants. All pregnant women who had a fetus with spina bifida (n = 15, study group) in the second trimester were included in the study. Nineteen (n = 19, control group) women who attended our clinic and had abnormal triple screens indicating an increased risk for Down's syndrome were included in the study as the control group. Detailed ultrasound (US) examination, fetal karyotyping, investigations for fetal cardiac malformations infections and genetic diseases were performed for all cases.

Mean maternal age was 27.3 ± 1.0 years for the spina bifida group and 28.0 ± 0.9 years for the control group. The mean gestational age at sampling was 19.2 ± 1.1 weeks for the spina bifida group and 18.8 ± 1.3 weeks for the control group. Obese patients and those with any systemic or endocrine disorder were excluded from the study. All pregnancies were accurately dated by the last menstrual period and by first-trimester US investigation. Amniotic fluid samples were obtained by routine transabdominal amniocentesis and collected into 10-ml dry tubes. All amniotic fluid samples were free of blood contamination and were immediately centrifuged at 3000 g for 10 min and stored at -20ºC until assayed. Levels of free amino acids (histidine, leucine, isoleucine, methionine, phenylalanine, tryptophan, valine, alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamine, glycine, tyrosine) were measured in amniotic fluid samples using EZ: fast kits (EZ: fast GC/FID free (physiological) amino acid kit) by gas chromatography (Focus GC AI 3000 Thermo Finnigan analyzer, Milan, Italy; injection: Split 1:15 at 250ºC, 2.5 µ; carrier gas: helium 1.5 ml/min (60 kPa) at 110ºC; pressure rise: 6 kPa/min; oven program: 30ºC/min from 110º to 320ºC, hold at 320º for 1 min; Detector: FID at 320ºC; intravariability: 2.4%; intervariability: 3.2%). The results are reported as means ± SD; a *t*-test was performed for statistical analysis. The statistical relationship between the two variables was checked by Pearson correlation coefficients. A p value of less than 0.05 was considered to be statistically significant.

Results

Fifteen women who had fetuses with spina bifida were included in the study. Spina bifida was diagnosed by US and confirmed after delivery. Detailed US examination, fetal karyotyping, and investigations for fetal cardiac malformation infections were performed for all cases. All investigations were normal. Pregnancy was terminated in the spina bifida group. The 19 control group women were

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submitted to amniocentesis performed because of abnormal triple screens indicating an increased risk for Down's syndrome. None of the control group fetuses showed structural abnormalities in US at the time of amniocentesis and none had chromosome abnormalities. All patients in the control group gave birth to a healthy child. The rates of nulliparity, the mean maternal and gestational ages and body mass index at the time of amniocentesis did not differ significantly between the two groups ($p < 0.05$).

The mean levels of alanine (37.2 ± 38.2 umol/l vs 15.5 ± 6.5 umol/l), cystathionine (14.2 ± 13.0 umol/l vs 13.1 ± 12.3 umol/l), cysteine (18.8 ± 9.5 umol/l vs 14.6 ± 5.5 umol/l), phenylalanine (56.2 ± 46.3 umol/l vs 43.3 ± 15.8 umol/l), tryptophane (80.8 ± 41.3 umol/l vs 44.3 ± 44.2 umol/l), tyrosine (44.5 ± 35.3 umol/l vs 22.2 ± 12.5 umol/l) amino acids were found to be significantly higher in fetuses with spina bifida than in the control group ($p < 0.05$).

Discussion

The pathogenesis of spina bifida remains unclear and depends on the underlying disorder. Alcohol consumption, maternal exposure to excess vitamin A, folic acid deficiency, and antiepileptic drugs are the common etiologies of spina bifida [1-4].

Emery *et al*. conducted a study to find out aminoacid concentrations of central nervous system malformations in 33 cases. The study showed that there was a significant increase in the amount of certain neutral amino acids such as methionine, isoleucine, leucine, tyrosine, and phenylalanine [3].

Pettit *et al*. compared amino acid concentrations in amniotic fluid from fetal neural tube defects and normal pregnancies and found that hydroxy amino acids were raised while the branched chain amino acids were lower in concentration [4].

We found that amino acid levels of alanine, cystathionine, cysteine, phenylalanine, tryptophane and tyrosine were significantly higher in fetuses of the control group than in the spina bifida group suggesting that loss of amino acids from the fetus through the spinal cord may contribute to the etiology of spina bifida. Emery *et al's* study found a significant increase in the amniotic fluid of tyrosine and phenylalanine amino acids as our study [3].

The loss of alanine, cystathionine, cysteine, phenylalanine, tryptophane and tyrosine amino acids from spinal cord to amniotic fluid might partly explain fetal morbidity and mortality.

This was a preliminary study on amniotic fluid amino acid concentrations conducted on a small patient series. We think that it would be beneficial to conduct further studies with larger groups to determine the amino acid levels of fetuses with spina bifida.

References

[1] Chen C.P.: "Prenatal diagnosis, fetal surgery, recurrence risk and differential diagnosis of neural tube defects". *Taiwan J. Obstet. Gynecol.,* 2008, *47,* 283.

[2] Sharland E., Montgomery B.D., Granell R.: "Folic acid in pregnancy - Is there a link with childhood asthma or wheeze?". *Aust. Fam. Physician*., 2011, *40,* 421.

[3] Emery A.E., Burt D., Scrimgeour J.B.: "Aminoacid composition of amniotic fluid in central nervous system malformations". *Lancet,* 1973, *301*, 970.

[4] Pettit B.R., Allen J.T.: "Comparison of amino acid concentrations in amniotic fluid from fetal neural tube defective and normal pregnancies". *Clin. Chim. Acta,* 1980, *104*, 43.

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