Amniotic fluid amino acid concentrations in fetal skeletal dysplasia

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Summary

The authors' objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with skeletal dysplasia in the second trimester of pregnancy. Eighteen pregnant women who had fetuses with with skeletal dysplasia detected by ultrasonography (skeletal dysplasia) in the second trimester and 35 women who had abnormal triple screenings indicating an increased risk for Down 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 and non-essential amino acids were measured in amniotic fluid samples using GC/FID free (physiological) amino acid kit by gas chromatography. The mean levels of essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) in amniotic fluid were found to be significantly lower in fetuses with skeletal dysplasia than in the control group ($p < 0.05$). The detection of significantly lower amino acid concentrations in the amniotic fluid of fetuses with a skeletal dysplasia compared to healthy fetuses suggests amino acid deficiency may play an etiological role in the pathogenesis of skeletal dysplasia.

Key words: Amino acids; Skeletal dysplasia; Amniotic fluid.

Introduction

Skeletal dysplasias are a group of congenital abnormalities of the bone and cartilage that are characterized by short stature. Skeletal dysplasia, sometimes called dwarfism, is a disorder of short stature defined as height that is three or more standard deviations below the mean height for age, race, and gender. Skeletal dysplasias involve disproportionately short stature, there are many other associated conditions such as short arms and truck, bowlegs, skull malformations, such as a large head, and cloverleaf skull. Maternal hydramnios is probably the most significant event associated with fetal skeletal dysplasia during pregnancy, and fetal hydrops is frequently observed. Fetal activity may be decreased in the lethal types of skeletal dysplasia [1].

The skeleton's mass is made up of non-living bone matrix and many tiny bone cells. Half of the bone matrix's mass is water, while the other half is collagen protein and solid crystals of calcium carbonate and calcium phosphate [2-3].

The aim of the present study was to determine the concentrations of amino acids in amniotic fluid of pregnant women whose fetuses were diagnosed to have skeletal dysplasia in the second trimester of pregnancy. The authors hypothesized that the concentrations of amino acids may be decreased in fetuses with skeletal dysplasia.

Materials and Methods

The study was performed at the Prenatal Diagnosis Unit of Dicle University Hospital between January 2010 and January 2013. The study was approved by the institutional review board and Ethics Committee of the university hospital, and written informed consent was obtained from all participants. All pregnant women who had a fetus with skeletal dysplasia (n = 18) in the second trimester were included in the study. The first 35 women who attended the present clinic and had abnormal triple screens indicating an increased risk for Down syndrome were included in the study as the control group (n = 35). Mean maternal age was 27.5 ± 2.3 years for the skeletal dysplasia group and 28.1 ± 3.4 years for the study group. The mean gestational age at sampling was 18.2 ± 1.1 weeks for the skeletal dysplasia group and 19.1 ± 1.3 weeks for the study group. Maternal body mass index was 29.2 ± 1.0 kg/m^2 in skeletal dysplasia group and 27.8 ± 1.2 kg/m^2 in the study group. Five women in the skeletal dysplasia group and seven in the control group were nulliparous (Table 1).

The authors evaluated biometric parameters and ultrasonography (U/S) findings consistent with the diagnosis of skeletal dysplasia. The femora, humeri, tibia, and ulna were symmetrically shortened. U/S scan performed and a decreased rate of development of the femora (femur length (FL) < 5th centile).

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 U/S 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. Venous blood samples were taken within ten minutes after amniocentesis from the pregnant women and collected into Ethylenediaminetetraacetic acid (EDTA)-containing tubes. All samples were immediately centrifuged at 3,000 *g* for ten minutes and stored at -20ºC until assayed. Levels of free amino acids (essential amino acids: histidine, leucine, lysine, isoleucine, methionine, phenylalanine, threonine, tryptophan, and valine) and non-essential amino acids (alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamic acid, glutamine, glycine, ornithine, and proline) were measured in plasma and amniotic fluid samples using GC/FID free (physiological) amino acid kit) by gas chromatography (focus GC

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Table 1. — Demographic characteristics of the study and control groups.

	Skeletal dysplasia group (n = 18)	Control group (n = 35)	*p* value
Maternal age (years)	27.5 ± 2.3	28.1 ± 3.4	0.376[b]
Nulliparity	5 (9.4%)	7 (13.2%)	0.805[b]
Gestational age at the time of amniocentesis (weeks)	18.2 ± 1.1	19.1 ± 1.3	0.447[b]
Maternal body mass index at the time of amniocentesis (kg/m²)	29.2 ± 1.0	27.8 ± 1.2	0.702[b]

Data are reported as means ± SD. There were no statistically significant differences between groups (Student *t*- test, $p > 0.05$).

Table 2. — Concentrations of 20 amino acids in amniotic fluid samples of fetuses with skeletal displasia and controls.

Amino acid	Control group (n = 35) (μmol/l)	Skeletal Dysplasia Group (n = 18) (μmol/l)	*p* value
Alanine	162.9 ± 49.2	158.2 ± 35.5	0.315
Asparagine	22.8 ± 3.9	23.5 ± 4.1	0.217
Aspartic acid	8.2 ± 1.2	8.1 ± 4.6	0.701
Cystathionine	2.6 ± 1.0	2.5 ± 1.4	0.439
Cysteine	29.5 ± 2.7	28.7 ± 3.3	0.101
Glutamic acid	33.5 ± 2.0	32.5 ± 6.3	0.605
Glutamine	42.0 ± 5.7	38.2 ± 9.9	0.508
Glycine	145.4 ± 41.9	142.3 ± 40.8	0.178
Histidine	57.3 ± 13.2	21.5 ± 4.8	0.002*
Isoleucine	18.2 ± 3.5	11.2 ± 1.1	0.013*
Leucine	75.1 ± 9.5	49.2 ± 8.8	0.019*
Lysine	92.5 ± 17.0	33.4 ± 5.5	0.002*
Methionine	29.8 ± 1.2	8.8 ±1.3	0.011*
Ornithine	20.1 ± 3.0	19.2 ± 2.9	0.197
Phenylalanine	41.3 ± 7.6	22.6 ± 4.9	0.012*
Proline	214.4 ± 28.8	218.5 ± 29.6	0.882
Threonine	132.6 ± 8.5	58.3 ± 10.7	0.015*
Tryptophan	18.7 ± 2.3	5.2 ± 1.2	0.018*
Tyrosine	55.2 ± 9.3	53.1 ± 7.0	0.778
Valine	171.4 ± 9.2	103.1 ± 11.4	0.002*

Data are reported as means ± SD. *$p < 0.05$ for the skeletal displasia group compared to control (Student *t*-test).

split 1:15 at 250ºC, 2.5 μ; carrier gas: helium 1.5 ml/min (60 kPa) at 110ºC; pressure rise: six kPa/min; oven program: 30ºC/minute from 110º to 320ºC, hold at 320º for one minute; 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

Eighteen women who had fetuses with skeletal dysplasia were included in the study (skeletal dysplasia group, n = 18). Skeletal dysplasia was diagnosed by U/S and confirmed after delivery. The chromosomal analysis of skeletal dysplasia group was normal. In the skeletal dysplasia group, all fetuses were terminated between 18-22 weeks of gestation with misoprostol induction.The control group consisted of 35 women submitted to amniocentesis performed because of abnormal triple screens indicating an increased risk for Down syndrome (control group, n = 35). None of the control group fetuses showed structural abnormalities in U/S at the time of amniocentesis and none had chromosome abnormalities. All patients in the control group gave birth to a healthy child. The characteristics of both groups of patients are shown in *Table 1*. 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 concentrations of amino acids in the skeletal dysplasia and control groups are shown in *Table 2.*

The mean concentrations of essential amino acids were significantly lower in the skeletal dysplasia group than in the control group ($p < 0.05$), whereas the mean concentrations of acidic amino acids (glutamine, glutamic acid, aspartic acid, asparagine), ornithine, and cystathionine did not differ statistically between groups ($p < 0.05$).

Discussion

Skeletal dysplasia is a term used to describe a range of more than 200 very rare conditions which affect cartilage and bone growth. Skeletal dysplasias are genetic diseases that interfere with normal bone development and growth. Skeletal dysplasias are lethal in the perinatal period, re-

stricting the growth of long bones and the resulting small thoracic cage causes a mechanical restriction of lung growth. This may be lethal at birth, or death may occur in early infancy. In general, the limb bones are markedly shortened and in perinatal lethal disease are often below the 5th percentile. The diagnosis of a lethal skeletal dysplasia can be assessed prenatally based on the short femur and small thorax [1].

The estimated frequency of lethal skeletal dysplasias is 1.5 per 10,000 births [2]. The most common lethal groups are osteogenesis imperfecta, thanatophoric dysplasias, achondrogenesis, and short ribbed polydactyly [3].

In many cases, there will already be a prenatal or neonatal diagnosis of a lethal skeletal dysplasia made before the autopsy is begun. The cornerstone of diagnosis is the radiograph. To an expert radiologist, the radiographs will usually establish the diagnosis of a specific type of skeletal dysplasia. In some cases, a molecular diagnosis will clarify doubts about the radiological diagnosis and render genetic counseling more secure [1-3].

Prenatal screening and diagnosis are primarily performed in the second trimester, but late first trimester fetal structural assessment is becoming more common with advances in transvaginal ultrasound imaging and the widespread use of first trimester screening for Down syndrome. Increased nuchal translucency can be associated with skeletal dysplasia; when this association is present, approximately 85 percent of cases are lethal skeletal dysplasias [4].

The abnormality seen in the bone of patients with skeletal dysplasia is failure of endochondral ossification. Intramembranous and periosteal ossification are undisturbed. Histologic studies have shown disarray of the chondrocytes, with loss of columnization and loss of normal chondrocyte proliferation. Endochondral growth is disturbed and the bones remain short [5].

Amino acids are small biological molecules that, when linked together in a long chain and folded into a globular structure, form a protein. Proteins serve both structural and physiological functions in the body. Of the 20 amino acids found in proteins, nine are essential to diet because body cannot produce them. When recovering from a bone injury, intake of essential amino acids becomes especially important. The development of in vitro cell culture methods has made it possible to study bone cell metabolism and growth and obtain a deeper insight into the pathophysiology of skeletal diseases. The studies support that amino acids has a positive effect on osteoblast proliferation, activation, and differentiation. Therefore, administration of amino acids may be useful in clinical treatment and prevention of skeletal disorders [6].

The present authors found lower levels of amniotic fluid essential amino acids in the skeletal dysplasia group than in the control group which might explain amino acid deficiency in fetuses with skeletal dysplasia. They speculate that replacement of essential amino acids might improve the outcome of infants with skeletal dysplasia during the neonatal period. This is a preliminary study on amniotic fluid amino acid concentrations conducted on a small patient series. They believe that it would be beneficial to conduct further studies with larger groups to determine the amino acid levels of fetuses with skeletal dysplasia.

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