Are alanine, cysteine, glycine and valine amino acids the cause of non-immune hydrops fetalis?

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Summary

Our objective was to measure amniotic fluid amino acid concentrations in pregnant women diagnosed as having fetuses with non immune hydrops fetalis in the second trimester of pregnancy. Twenty-three pregnant women who had fetuses with non immune hydrops fetalis detected by ultrasonography (non immune hydrops fetalis group) in the second trimester and 19 women who 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, cysteine, glycine and valine amino acids were found to be significantly higher in fetuses with non immune hydrops fetalis than in the control group ($p < 0.05$). The detection of significantly higher amino acid concentrations in the amniotic fluid of fetuses with a non immune hydrops fetalis in healthy fetuses suggests loss of amino acids from the fetus through capillary permeability or/and the lymphatic system through the amniotic fluid may contribute to the etiology of non-immune hydrops fetalis.

Key words: Non-immune hydrops fetalis; Amniocentesis.

Introduction

Hydrops fetalis is a medical condition characterized by an accumulation of fluid, or edema, in at least two fetal compartments. Immune and non-immune are two types of hydrops fetalis [1]. Hydrops is classified as nonimmune hydrops fetalis (NIHF) when it occurs without evidence of isoimmunization. NIHF is an uncommon but serious disorder associated with an overall poor prognosis. There are more than 100 causes of NIHF, but cardiac and functional anomalies are a common cause. However the success rate of prenatally diagnosed NIHF in finding an underlying cause may be as low as 40% [2]. The aim of our study was to determine the amino acid concentrations of NIHF 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 the other causes of NIHF.

Material and Methods

The study was performed at the Prenatal Diagnosis Unit of Dicle University Hospital and Adana Numune Research Hospital between January 2009 and June 2011. 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 NIHF (n = 23, 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 should be performed for all cases

Mean maternal age was 28.2 ± 1.0 years for the NIHF group and 29.0 ± 1.1 years for the control group. The mean gestational age at sampling was 19.1 ± 1.3 weeks for the NIHF group and 18.9 ± 1.0 weeks for the control group. Maternal body mass index was 24.3 ± 1.0 kg/m^2 in the NIHF group and 25.2 ± 1.0 kg/m^2 in 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. All samples 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, and valine, alanine, asparagine, aspartic acid, cystathionine, cysteine, glutamine, glycine, tyrosine) were measured in plasma and 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

Twenty-three women who had fetuses with NIHF were included in the study (non-immune hydrops fetalis group). NIHF was diagnosed by US and confirmed after delivery. Detailed US examination, fetal karyotyping, and investigations for fetal cardiac malformation infections

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were performed for all cases. All investigations were normal. Pregnancy was terminated in the NIHF group. The 19 control group women were submitted to amniocentesis 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 concentrations of alanine (424.9 ± 290.7 vs 392.5 ± 83.1) cysteine (30.3 ± 19.1 vs 14.6 ± 5.5) glycine (322.1 ± 242.3 vs 209.0 ± 82.0) and valine (239.1 ± 69.8 versus 199.5±109.8) amino acids were significantly higher in the NIHF group than in the control group ($p < 0.05$).

Discussion

The pathogenesis of NIHF remains unclear and depends on the underlying disorder. Reduction in osmotic pressure due to liver disease or nephropathy may be a cause. The common etiologies of NIHF include cardiogenic and chromosal anomalies, viral infections, and fetal anemia [3].

The cardiovascular group includes structural abnormalities, arrhytmias, and myocardiopathies. The pathophysiology of structural cardiac anomalies leading to hydrops is high right atrial pressure, or volume overload, and right heart congestion resulting in increased central venous pressure, and heart failure leads to edema [4].

Hypoproteinemia and liver dysfunction can result in a low osmotic pressure causing leakage of fluid into the interstitium due to lower osmotic pressure of the intravascular compartment in NIHF cases [5].

The underlying mechanism for hydrops is still not clear. The link between a cause and mechanism that generates hydrops is not always clear. The fetus is at risk of interstitial fluid accumulation owing to great capillary permeability and lymphatic disorders [6].

We found that amino acid levels of alanine, cysteine, glycine, and valine amino acids in amniotic fluid were significantly higher in the NIHF group than in the control group suggesting that loss of amino acids from the fetus through capillary permeability or/and lymphatic system through the amniotic fluid may contribute to the etiology of NIHF. The causes of cardiogenic and chromosal anomalies, viral infections and fetal anemia may be complicated by alanine, cysteine, glycine, and valine amino acid loss which might partly explain fetal morbidity and mortality.

This is 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 non-immune hydrops fetalis.

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