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Plasma Atrial Natriuretic Peptide in Fetuses with Cardiac Disease

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Key Words

Atrial natriuretic peptide · Polyhydramnios · Cardiac disease in fetuses · Fetal heart disease

Abstract

Objective: To investigate atrial natriuretic peptide (ANP) levels in fetuses with cardiac defects and to evaluate the relationships between plasma ANP levels and the presence of polyhydramnios. ***Methods:*** Plasma ANP levels were measured by radioimmunoassay in 27 fetuses with cardiac abnormalities and in 14 normal healthy fetuses. ***Results:*** Fetal plasma ANP levels were similar in the two studied groups ($p = 0.18$) but they were significantly higher in a subset of cases with cardiac disease and polyhydramnios ($n = 7$) than in those with cardiac disease and normal amniotic fluid ($n = 20$; $p = 0.036$) and controls ($p = 0.01$). ***Conclusion:*** Polyhydramnios in fetuses with heart conditions might be explained by increased fetal diuresis secondary to increased ANP production.

Introduction

Atrial natriuretic peptide (ANP) is a member of a family of natriuretic peptides that regulate blood pressure and body fluid homeostasis through their diuretic, natriuretic and vasorelaxant effects [1, 2]. These peptides also modulate cardiac hypertrophy by regulating apoptosis in myocytes [3]. During fetal development atrial myocytes are the predominant source of ANP production, but (unlike in adults) significant amounts are also produced by ventricular myocytes [4]. The primary acute stimulus for release is increased stretch of the myocardium, but the secretion is also stimulated by volume loading, hyperosmolality, hypoxia and response to vasoconstrictors.

This peptide has been involved in the pathogenesis of fetal morbidity associated with several conditions. In maternal diabetes, increased fetal ANP production has been related to both fetal cardiac hypertrophy and polyhydramnios [5]. In twin-to-twin transfusion syndrome, the levels of ANP correlated with the degree of polyhydramnios [6] and cardiac dysfunction in the recipient [7]. In immune and non-immune fetal hydrops, circulating ANP levels have been found to be increased [8, 9]. In children with congenital heart disease, ANP has been related to important haemodynamic variables, such as atrial and pulmonary artery mean pressure [10, 11], and ANP has been suggested to be an important factor in pulmonary hypertension [12]. It has also been related to symptoms of heart failure [11]. However, little is known about the relationships between ANP and cardiac disease during fetal life.

The aim of the present study was to investigate ANP levels in fetuses with cardiac defects and to evaluate the relationships between plasma ANP levels and the presence of polyhydramnios.

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Table 1. Demographics of the studied groups

Parameter	Cardiac abnormality (n = 27)	Control (n = 14)	p
Gestational age at FBS, weeks	21 (20.75–22)	22 (20–23)	0.32
Maternal age, years	30 ± 6.5	32.9 ± 6.5	0.18
Previous pregnancies	2 (1.75–3)	2 (1–3)	0.45
Parity	1 (0.75–1.25)	1 (0–1)	0.33

Results are means with interquartile ranges in parentheses. FBS = Fetal blood sampling.

Subjects and Methods

Plasma ANP levels were studied in 27 fetuses with cardiac abnormalities and in 14 normal healthy fetuses who underwent fetal blood sampling for clinical indications. After consent as approved by the local ethics committee, umbilical cord blood in surplus to the diagnostic need was collected by ultrasound-guided fetal blood sampling and transferred to 3-ml silicone-coated blood-collecting tubes containing heparin. The blood was centrifuged for 20 min at 2,000 *g*. Aliquots of the plasma were then stored at –20°C until required for assay. The cardiac malformations were ventricular septal defect (n = 8), atrioventricular septal defect (n = 7), hypoplastic left heart (n = 5), hypoplastic right heart (n = 3), tetralogy of Fallot (n = 2), pulmonary stenosis (n = 1) and univentricular heart (n = 1). The associated anomalies in cardiac cases were chromosomal abnormalities (n = 8) (trisomy 21: n = 5; trisomy 18: n = 2; delection chromosome 22: n = 1) and structural abnormalities (n = 5) (bowel obstruction: n = 1; short bones: n = 1; exomphalos: n = 1; talipes: n = 1, and absent radius: n = 1).

Diagnoses were done on the bases of ultrasound findings, postmortem and/or neonatal investigations. The indications for fetal blood sampling in the control group were abnormal serum screening (n = 3), sonographic 'markers' (increased nuchal thickness: n = 1; mild renal dilatation: n = 2; hyperechogenic bowel: n = 2; choroid plexus cyst: n = 2; echogenic foci in the heart: n = 2; strawberry-shaped head: n = 1), previous child affected (n = 2), suspected chromosomal mosaicism (n = 1), maternal age/late booking (n = 2).

ANP plasma levels were measured by radioimmunoassay as previously described [13]. Briefly, ANP was extracted from plasma using OD S-Silica (Sep-Pak C18 columns; Waters Associates, Milford, Mass., USA) and assayed using a monoclonal antibody within a radioimmunoassay system. The overall recovery of ANP (± SD) was 94.8% (5.9) with a detection limit of 1 pg/ml. Using previously reported data [14, 15], we calculated that a sample of 26 cases and 13 controls was needed to show a difference with an α level of 0.05 and a power $(1 - \beta)$ of 80%. We enrolled 1 more woman in each group to account for withdrawals. The distribution of the variables was checked using histograms and Kolmogorov-Smirnov tests. Results are given as mean ± SD for normal variables and as mean (interquartile range) for non-parametric variables. We used Student t test and Mann-Whitney U test for comparisons between groups for normally and non-normally distributed variables, respectively. Since ANP was non-normally distributed, Kruskal-Wallis test was used for multiple subgroup comparisons, and post-hoc differences among subgroups were studied by adjusted Mann-Whitney U test. Correlations were studied using Spearman correlation coefficient. Linear regression was used to find the best predictive model between variables and ANP (using log-transformed values). Statistical significance was set at $p < 0.05$.

Results

There were no differences in demographics between the two studied groups (table 1). Fourteen women from the cardiac abnormality group (51.9%) opted for termination of pregnancy, 7 (25.9%) had induction of labour at term and normal vaginal delivery, 2 underwent emergency caesarean section (7.4%), 2 had elective caesarean section (7.4%) and 1 had spontaneous vaginal delivery (3.7%). There was no significant correlation between fetal plasma ANP levels and maternal age, number of previous gestations, parity, gestational age at the time of diagnosis or fetal blood sampling, gestational age at delivery or birth weight, and Apgar scores in either of the studied groups.

Fetal plasma ANP levels were similar between the two groups: controls 29 (8.75–37.75) vs. cardiac group 36 (17–69) pg/ml ($p = 0.18$). Moreover, the levels of ANP were not significantly different according to the type of cardiac malformation: atrioventricular septal defect 30 (12–76) pg/ml, tetralogy of Fallot 44 (19–44) pg/ml, hypoplastic left heart 15 (7.5–46) pg/ml, hypoplastic right heart 21 (17–21) pg/ml, ventricular septal defect 48.1 (30–315) pg/ml, pulmonary stenosis 152 pg/ml and univentricular heart 53 pg/ml ($p = 0.36$). In order to simplify the analysis and the graphic information, a simplified classification in 3 groups (controls and cardiac disease with or without ventricular septal defect, in other words uni- or biventricular congenital heart disease) was used (fig. 1). However there were 7 fetuses with cardiac defects whose plasma ANP levels were higher than the 95th centile value in the control group (58 pg/ml). They

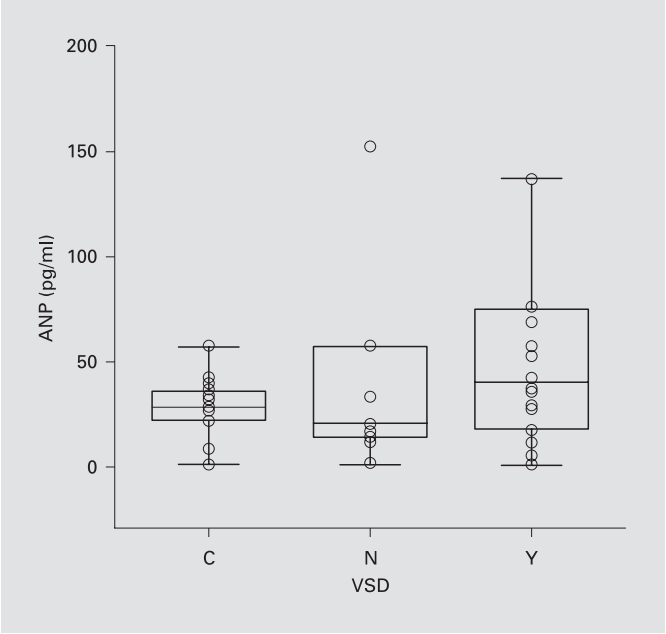

Fig. 1. Fetal ANP levels according to the presence (group Y) or absence (N) of ventricular septal defect. Group Y includes cases with ventricular septal defect (n = 8), atrioventricular septal defect (n = 7), tetralogy of Fallot (n = 2), and univentricular heart (n = 1). Group N includes cases with hypoplastic left heart (n = 5), hypoplastic right heart (n = 3), and pulmonary stenosis (n = 1). The control group is shown as group C. Two extreme values, both in the group Y, have been omitted from the graph to improve the graphic key information. p = 0.27 for intergroup differences.

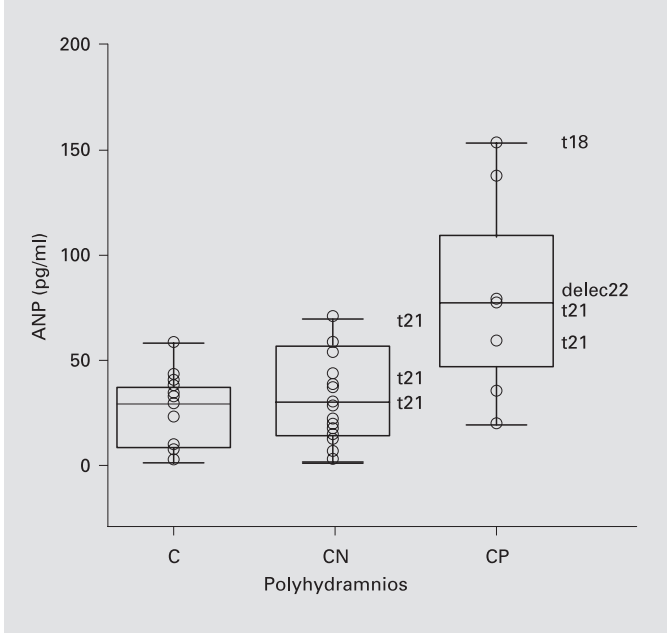

Fig. 2. Fetal ANP levels according to the presence or absence of polyhydramnios. C = Controls (n = 14); CN = cardiac defects with normal amniotic fluid (n = 20); CP = cardiac defects and polyhydramnios (n = 7). Cases with chromosomal abnormalities are labelled in the graph. Two extreme values, both in the group CN (one of them a trisomy 18 case) have been omitted from the graph to improve the graphic key information. p = 0.04 for intergroup differences. p = 0.03 for the difference between group CP and group CN; p = 0.01 for the difference between group CP and group C.

were cases of ventricular septal defects (n = 3), atrioventricular septal defect (n = 2), tetralogy of Fallot (n = 1) and pulmonary stenosis (n = 1). Five of them (71.4%) had a chromosomal abnormality (2 cases of trisomy 21, 2 cases of trisomy 18 and 1 of chromosome 22 deletion).

There were 2 cases showing ultrasound signs of cardiac failure. One of them had ventricular septal defect and ascites and the ANP level was normal (36 pg/ml). The other had a different ventricular septal defect, in this case associated with complete heart block and hydrops and marked by elevated ANP levels (795 pg/ml).

Five of the 7 cases (72.4%) with increased ANP levels had polyhydramnios. ANP levels were significantly higher in the 7 cases with cardiac disease and polyhydramnios [76 (34–136.3) pg/ml] than in those with cardiac disease and normal amniotic fluid (n = 20) [29 (12.75–56.37) pg/ml; p = 0.036] and in controls (p = 0.01; p = 0.04 for intergroup difference) (fig. 2). There was no difference in ANP levels between the control group and the group of fetuses with heart defects and normal amniotic fluid (p = 0.57). There were not significant differences in cardiac abnormalities between fetuses with polyhydramnios and those with normal amniotic fluid volume (p = 0.54), as shown in table 2. Other structural abnormalities were distributed as follows: the case with bowel obstruction and the one with short bones were in the polyhydramnios group and the cases with exomphalos, talipes and absent radius were in the normal amniotic fluid volume group. There were only 2 cases of polyhydramnios and normal ANP levels. One had tetralogy of Fallot with associated bowel obstruction, the other was a case of dextrocardia and hypoplastic left heart.

Table 2. Distribution of cardiac defects according to presence or absence of polyhydramnios

Type of defect	Normal amniotic fluid (n = 20)	Polyhydramnios (n = 7)
Atrioventricular septal defect	5 (25%)	2 (28.6%)
Tetralogy of Fallot	1 (5%)	1 (14.3%)
Hypoplastic left heart	4 (20%)	1 (14.3%)
Hypoplastic right heart	3 (15%)	0
Pulmonary stenosis	0	1 (14.3%)
Univentricular heart	1 (5%)	0
Ventricular septal defect	6 (30%)	2 (28.6%)

Groups did not differ significantly (p = 0.54).

Discussion

It has been reported that fetal plasma ANP is unchanged during gestation [8] *and* that cardiac gene expression of ANP decreases during pregnancy [16, 17]. We did not find significant correlations between ANP levels and gestational age in either controls or cardiac patients. In another study of amniotic fluid ANP, levels were increased in cases of fetal cardiac malformations in fetuses with or without chromosomal abnormalities [18]. We compared fetuses with cardiac disease and controls and we found that, as a group, fetuses with cardiac disease did not have increased ANP levels. However, the levels varied widely from one case to another and there were particular cases in which the levels were very increased.

In children aged between 1 month and 15 years, ANP was much higher in those with ventricular septal defects than in children with other malformations, and the levels were normal in cases of tetralogy of Fallot [19]. We could not find statistically significant differences in ANP levels according to the type of malformation, even though the numbers in some groups were too small. The primary determinant of ANP seems to be an increased intracardiac volume or pressure, not the type of defect. Similarly, atriuretic peptides have been proposed to be useful to detect patients with heart failure [20]. As we did not measure intracardiac pressure and as most mothers of patients with increased ANP levels opted for termination of pregnancy, we could not get any follow-up data to evaluate whether or not these fetuses would develop signs of cardiac failure. We found 2 cases with ultrasound signs of cardiac failure but only 1 had increased levels of ANP.

Four of the fetuses with raised ANP levels had chromosomal abnormalities. In a previous report, Hyett et al. [16] found increased ANP gene expression in the heart of trisomic fetuses, and it was hypothesised that these increased levels should be interpreted as a sign of cardiac failure. Further studies are needed to evaluate circulating ANP levels in chromosomically abnormal fetuses by studying cases with or without cardiac abnormalities separately.

Increased ANP levels have been involved in the origin of polyhydramnios in different situations such as maternal diabetes or twin-to-twin transfusion syndrome. We found increased levels in cases with cardiac defects plus polyhydramnios. There were only 2 cases in who polyhydramnios could not be explained by increased ANP levels. One of them had bowel obstruction, which is a cause of polyhydramnios itself. The other had a hypoplastic left heart and dextrocardia, and it may be speculated that the abnormal situation of the heart could contribute to the origin of the polyhydramnios via mechanical compression of the oesophagus. Our results suggest that if no other obvious cause of polyhydramnios can be seen, the presence of increased amniotic fluid volume associated with congenital heart defect is caused by increased fetal diuresis secondary to increased ANP production. Therefore the presence of polyhydramnios in fetuses with cardiac disease may be related to an increased presence of the hemodynamic determinants of plasma ANP concentrations, such as right atrial area, right atrial pressure and pulmonary resistance [12, 21]. Particularly the presence of pulmonary hypertension has major clinical implications, not only in cases of cardiac disease but also in other congenital malformations such as congenital diaphragmatic hernia or skeletal dysplasias, where polyhydramnios is also a frequent finding. Further studies are needed to evaluate the relationships between amniotic fluid volume and pulmonary artery blood flow resistance in fetuses with these conditions.

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