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CLINICAL INVESTIGATION

Correlation Between Down Syndrome and the Level of Placental Alkaline Phosphatase in Amniotic Fluid

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Abstract: The aim of this study was to investigate the correlation between Down syndrome and the level of placental alkaline phosphatase (PLAP) in amniotic fluid. A total of 279 amniotic fluid samples taken between 14 and 24 gestational weeks were investigated in this study. Karyotype analysis was made in all samples. In 10 samples trisomy 21 was determined, in one sample trisomy 18, in two samples 47,XXY, in six samples various structural chromosomal anomalies and in 260 samples normal karyotype. PLAP was measured using a fluorometric heat inactivation assay. Mean PLAP multiples of normal gestational median (MOM) value in samples with Down syndrome was 0.44 MOM, and in those with normal karyotype was 1.14 MOM. In fetuses with Down syndrome, amniotic fluid PLAP levels were significantly reduced ($p = 0.002$). There was no difference between mean PLAP MOM values in samples with normal karyotype and in those with structural chromosomal abnormality ($p = 0.47$). When 0.37 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid, detection rate (sensitivity) of the test was 50%, specificity was 94%, and false-positive rate was 5.8%. We conclude that PLAP levels are reduced in the amniotic fluid of women carrying a fetus with Down syndrome.

Key Words: PLAP (placental alkaline phosphatase), Down syndrome, amniotic fluid.

Introduction

Down syndrome (trisomy 21) is the most common chromosomal abnormality and causes severe mental retardation (1,2). The frequency of occurrence is estimated to be 1:700 live births (2). The risk is particularly high in mothers aged 35 years and older.

Currently, risk calculation for Down syndrome based on maternal age in combination with the determination of alpha-fetoprotein, estriol and human chorionic gonadotropin in maternal serum (known as "triple marker screen") provides a detection rate of 60% of all cases with trisomy 21 and a false-positive rate of 5% (3,4). For given multiples of the median (MOM) for the three markers, however, the calculated risk is exclusively dependent on the maternal age. As a consequence, the sensitivity of serum screening is much better in women ≥ 35 years than in younger women (3). The most certain method for prenatal diagnosis of Down syndrome is amniocentesis and fetal chromosomal analysis (5). However amniocentesis cannot be performed in all pregnancies. Because women ≥ 35 years of age carry the highest risk of a fetus with Down syndrome, amniocentesis is usually done in these cases. However, 70-80% of children with Down syndrome have been delivered by women < 35 years of age (6,7). In addition, fetal chromosomal analysis is expensive, difficult and time-consuming. Thus, more cost-effective, simple, and safe new methods should be investigated.

The aim of this study was to investigate the correlation between a fetus with Down syndrome and the level of placental alkaline phosphatase (PLAP) in amniotic fluid.

Material and Method

Our study population consisted of 273 women (268 single, 4 twin, 1 triple pregnancy) who attended the Department of Pediatrics, Clinical Genetics Unit, Hacettepe University Faculty of Medicine for prenatal diagnosis. After obtaining informed consent from the women, a total of 279 amniotic fluid samples were taken. Maternal age was calculated in years according to the birth date. Gestational age was calculated in weeks according to the date of the last menstrual period, and was confirmed by ultrasound.

A total of 279 amniotic fluid samples were obtained from mothers aged between 20-46 years (mean maternal age: 34.4 ± 5.1 years). Sixty-six percent of mothers were 35 years or older. Amniocentesis was performed between the 14th and 24th gestational week (mean gestational age at the time was 18.46±1.9 weeks).

There were seven distinctive indications for amniocentesis: maternal age ≥ 35 years (184 samples, 66%), history of a previous pregnancy in which chromosomal anomalies were determined in the fetus (25 samples, 9%), history of a previous child with mental-motor retardation and/or congenital anomalies (18 samples, 6.4%), positive triple serum screening test (Down syndrome risk of 1 in 250 or higher) (23 samples, 8.2%), abnormal fetal ultrasound (oligohydramnios, hydrops fetalis, ventriculomegaly, intrauterine growth retardation, single umbilical artery, choroid plexus cyst, thickened nuchal fold, short femur) (15 samples, 5.4%), history of a previous child with a disease for which amniocentesis was done for prenatal diagnosis (cystic fibrosis, galactosemia, adrenogenital syndrome, Krabbe's disease, Hurler's syndrome) (7 samples, 2.5%), and other reasons for amniocentesis (recurrent miscarriage, miscarriage imminence, history of stillbirth) (7 samples, 2.5%).

After 20 ml of amniotic fluid was obtained, samples were centrifuged at 1000 rpm for 10 minutes in a refrigerated centrifuge. The pellet was recovered and used for cell cultures. Fluid supernatants were used for biochemical analysis after storage at –20 °C.

Amniotic cell culture: Amniotic fluid cells were harvested in the culture vessels using Chromosome Diagnostic Medium. After seven or ten days, karyotypes were prepared from metaphases banded with Giemsa-Tripsin-G method. For each sample, karyotype analysis was made in twenty metaphases.

PLAP assay: Stored amniotic fluid samples were thawed at room temperature. Total alkaline phosphatase activity of samples was determined using BM/Hitachi 911 model autoanalysatory by Boehringer Manheim kit. All assays were made in duplicate and activity expressed as units per liter of sample. Then, for thermal inactivation, 0.5 ml of amniotic fluid was incubated in a water bath at 65 °C for 30 minutes, after which the samples were rapidly cooled to room temperature. After being centrifuged at 3000 Xg for 15 minutes, supernatants were separated. Residual alkaline phosphatase activity was measured as described above. Because other alkaline phosphatase isoenzymes were inactivated at 65 °C for 30 minutes, the last determined heat stable alkaline phosphatase activity demonstrates PLAP activity (8-11).

PLAP values in the amniotic fluid of normal karyotype pregnancies were presented as medians for each week of pregnancy. PLAP values in the amniotic fluid of Down syndrome pregnancies were expressed in multiples of the median (MOM) value of the normal karyotype group of the same gestation. The medians for Down syndrome and normal karyotypes were compared using Student's t-test and Fisher's exact k-square test. The difference was considered significant when p value was < 0.05. Detection rate (sensitivity) and false-positive rates (1-specificity) were calculated (12).

Results

In this study, karyotyping was done for all cases who underwent amniocentesis. In 260 samples (93.2%) normal karyotype (46,XX or 46,XY) was demonstrated. In 10 samples (3.6%) trisomy 21 (47,XX,+21 or 47,XY,+21) was determined, in one sample (0.4%) trisomy 18 (47,XX,+18), and in two samples (0.7%) 47,XXY. In six samples (2.1%), various structural chromosomal anomalies were found: in one sample each 46,XX,t(12;21), 46,XX,t(2;21), 46,XY,t(4;8), and 46,XX,13p+, and in two samples 46,XY,inv(9).

The mean age of the mothers who carried a baby with normal karyotype was 34.1 ± 5.0 years, and with Down syndrome was 39.1 ± 3.7 years. There was a statistically significant difference between the two groups ($p = 0.002$). Nine of the 10 mothers who carried a baby with Down syndrome were 35 years or older. The other mother was 34 years old.

Mean gestational age in cases of mothers who carried a baby with normal karyotype was 18.51±1.9 weeks,

and with Down syndrome was 17.7 ± 2.1 weeks. There was no statistical difference between the two groups (p = 0.187).

Mean total alkaline phosphatase activity of all samples was 44.85 U/L ± 30.43. No difference was found with respect to mean total alkaline phosphatase activity between samples with Down syndrome and those with normal karyotype (p = 0.325).

Table 1 shows distribution of PLAP activity as mean ± standard deviation, median and MOM in all amniotic fluid samples and in samples with Down syndrome according to gestational weeks. From 16 to 20 weeks of gestation, there was a rise in the level of PLAP activity. PLAP levels declined at 20-21 weeks and then repeatedly increased. Table 2 demonstrates amniotic fluid PLAP activity as mean ± standard deviation, and mean PLAP MOM in normal karyotypes, Down syndrome and structural chromosomal anomalies groups.

Figure 1 shows distribution of PLAP activity as MOM in amniotic fluid samples with Down syndrome, trisomy 18, 47,XXY and other structural chromosomal anomalies according to gestational weeks. This figure showed that most of the PLAP MOM values in samples with Down syndrome were 0.5 MOM or less. When 0.5 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid, there was a statistically significant difference between the normal karyotype and Down syndrome groups (P < 0.0001).

When 0.5 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid, detection rate (sensitivity) of the test was 80%, specificity was 94%, and false-positive rate was 15%. Due to the insufficient number of

Table 1. Distribution of PLAP activity as mean ± standard deviation, median and MOM (multiples of the normal median) in all amniotic fluid samples and samples with Down syndrome according to gestational weeks.

Gestation week	Number of samples (%)	Mean PLAP (U/L) ± SD	Median PLAP (U/L)	Number of Down syndrome	MOM according to gestation week
14	2 (0.7)	2.55	2.55	1	0.94
15	3 (1.1)	4.14 ± 4.16	1.86	0	-
16	41 (14.7)	3.05 ± 1.56	2.68	2	0.11 and 0.50
17	46 (16.5)	3.14 ± 1.80	2.86	2	0.37 and 0.38
18	64 (22.9)	3.55 ± 1.81	3.11	1	0.64
19	42 (15)	3.30 ± 1.47	2.97	2	0.35 and 0.21
20	37 (13.3)	3.54 ± 1.88	3.31	1	0.47
21	21 (7.5)	3.16 ± 1.16	2.86	1	0.45
22	19 (6.8)	4.02 ± 1.35	3.73	0	-
23	3 (1.1)	4.14 ± 1.63	4.11	0	-
24	1 (0.4)	4.40	4.40	0	-
Total	279 (100)	3.45 ± 2.29	-	10	-

Table 2. Amniotic PLAP activity as mean ± standard deviation, and mean PLAP MOM in normal karyotypes, Down syndrome and structural chromosomal anomalies groups.

	Mean PLAP (U/L) ± SD	P value*	Mean PLAP MOM value	P value*
Normal karyotype n = 260	3.55 ± 2.32		1.14	
Down syndrome n = 10	1.26 ± 0.61	0.002	0.44	0.002
Structural chromosomal anomalies n = 6	2.32 ± 1.92	0.24	0.92	0.47

*The p values refers to statistical differences between each disease group and the normal karyotype group.

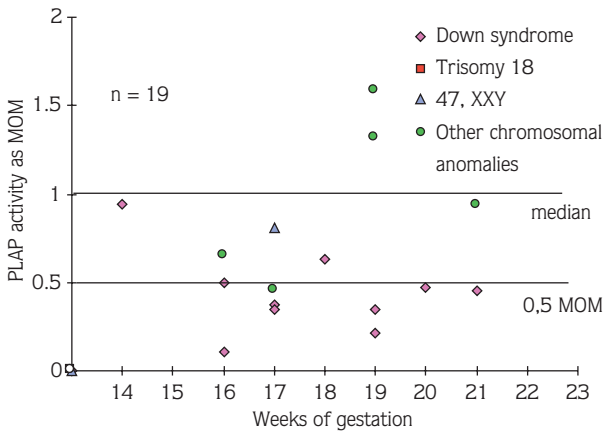

Figure 1. Distribution of PLAP activity as MOM (multiples of the normal median) in amniotic fluid samples with Down syndrome, trisomy 18, 47,XXY and other structural chromosomal anomalies according to gestational weeks.

samples, when other gestational weeks were excluded from the statistical analysis, sensitivity of the test between 16 and 21 gestational weeks was 89%. When 0.37 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid in order to achieve a lower false-positive rate, detection rate of the test was 50%, specificity was 94%, and the false-positive rate fell to 5.8%.

Discussion

Second trimester PLAP has recently been investigated as a new amniotic fluid prenatal screening method for Down syndrome (11,13,14). Ind et al. (13) showed for the first time in 1993 that PLAP activity in amniotic fluid may be used for prenatal screening of Down syndrome. Following their report, other studies confirmed the results of the study that PLAP levels were reduced in the amniotic fluid of women carrying a fetus with trisomy 21 (11,13,14). The cause of the decline at 20-21 weeks may be dilutional because of the increase in amniotic fluid.

In previous studies, median PLAP MOM in amniotic fluid of fetuses with Down syndrome was 0.63 and 0.72 MOM (12,13). In our study, between 14 and 21 gestational weeks, the median PLAP MOM in amniotic fluid with Down syndrome was 0.42 MOM; between 16-21 gestational weeks the value was 0.38 MOM. Our results are significantly lower than previous results.

For prenatal screening of Down syndrome, a cut-off value of PLAP activity in amniotic fluid has not been reported in the literature. According to our report, when 0.5 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid, detection rate of the test was 80%, specificity was 94%, and the false-positive rate was 15%. Due to the insufficient number of samples, when other gestational weeks were excluded from the statistical analysis, sensitivity of the test between 16 and 21 gestational weeks was 89%. For a lower false-positive rate, when 0.37 MOM was accepted as a cut-off value of PLAP activity in amniotic fluid, detection rate of the test was 50%, specificity was 94%, and false-positive rate was 5.8%. When these results were compared individually with alpha-fetoprotein, estriol and human chorionic gonadotropin levels in maternal serum, the detection rate was higher than with alpha-fetoprotein and estriol, but was lower than with human chorionic gonadotropin (15-17).

In conclusion, we showed in our study that PLAP levels are reduced in the amniotic fluid of women carrying a fetus with Down syndrome. Measurement of PLAP activity is a simple, cheap and quick method. However, amniocentesis is an invasive, difficult, and risky method requiring a specialist. For this reason, during the first and second trimester, maternal serum PLAP activity has been investigated as a prenatal screening method for Down syndrome. No significant difference was found for PLAP activity in maternal serum between fetuses with Down syndrome and those with normal karyotype (10,18-21). Also, Newby et al. (19) showed that there was correlation between maternal serum and placental extract levels of PLAP with Down syndrome. This relationship between placental and maternal serum PLAP levels suggests that transport of PLAP from the site of synthesis to the maternal circulation is not deranged in Down syndrome pregnancies, although reduction in amniotic fluid may be due to impaired transport from placenta to amniotic fluid. Denier et al. (22) reported that there was over-expression of the placental isozyme compared with the tissue-nonspecific form in neutrophils of mother with a trisomy 21 fetus.

PLAP is a microvillar enzyme produced by the syncytiotrophoblast of the human placenta. It is a membrane bound molecule which acts as a receptor for the Fc portion of immunoglobulin G. Thus PLAP plays a key role in the transport of IgG to the human fetus (23).

The explanation for reduced PLAP levels in amniotic fluid of women carrying a fetus with Down syndrome is not known, but it may be due to fetuses with Down syndrome and their placentas being immature, an explanation we have suggested for the low alpha-fetoprotein and estriol concentrations found in affected pregnancies.

Further studies with larger groups should be performed to investigate the mechanism for decreased PLAP levels in amnioic fluid in the Down syndrome fetus and the correlation with other markers (such as maternal age), which are important in screening for Down syndrome. Addition of new parameters for Down syndrome might improve the effectiveness of antenatal screening.

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