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A disintegrin and metalloproteinase domain-containing protein-12 levels in first-trimester pregnant women

M. Durmuscan[1], S. Yildirmak[2], V. Mihmanli[3], Y. Gulen Cicek[4], M. Vardar[5], F. Sezgin[6], O. Dikker[5], B. Basat[5]

[1]Department of Clinical Biochemistry, Public Health Laboratory, Adana
[2]Department of Clinical Biochemistry, Giresun University Medical Faculty, Giresun
[4]Department of Clinical Biochemistry, Yerkoy State Hospital, Yozgat
[3]Department of Obstetric and Gynecology and [5]Department of Clinical Biochemistry, SB Okmeydani Educational and Research Hospital, Istanbul
[6]Department of Industrial Engeneering, Istanbul University, Istanbul (Turkey)

Summary

Aim: In this prospective monocenter study, the authors aimed to investigate the correlations between levels of serum human disintegrin and metalloproteinase domain-containing protein 12 (ADAM12), pregnancy-associated plasma protein A (PAPP-A), serum-free beta hCG (fβ-hCG), and baby birth weights in two groups of pregnant women whose risks for trisomy 21 was found higher and lower than threshold value in first trimester screening test. *Materials and Methods:* Seventy-nine pregnant women were included the study. Using first trimester screening test, 40 of them were categorized as having above threshold risk (1/250) for trisomy 21 and, 39 of them were below threshold risk. ELISA method was used to measure the levels of serum ADAM12 and chemiluminescence method was used to measure the levels of PAPP-A and fβ-hCG. *Results:* In pregnant women at risk, ADAM12, PAPP-A multiple of median (MoM), and baby birth weights were found significantly lower than control group ($p < 0.001$, $p < 0.001$, and $p = 0.029$, respectively) while the levels of fβ-hCG MoM were higher than those of control group ($p < 0.001$). In the group of pregnant women having low birth weight (LBW) babies, ADAM12 levels were found lower than the group having normal birth weight babies (NBW) ($p < 0.033$). Also, the values of fβ-hCG MoM were found higher in comparison to NBW group ($p < 0.029$). A positive significant correlation was observed between ADAM12 concentrations and PAPP-A MoM ($r = 0.630$). *Conclusions:* Maternal serum ADAM12 levels are useful as biomarkers which support other screening parameters for predicting trisomy 21 risk. Additionally, maternal serum ADAM12 levels could be used for prediction of baby birth weights.

Key words: First-trimester screening test; A disintegrin and metalloproteinase domain-containing protein 12; Placenta associated plasma protein-A; Free beta-hCG.

Introduction

Early identification of high risk pregnancies is crucial to save time for more comprehensive tests and to provide more alternatives for decision concerning the *future* of the *fetus* [1]. There have been certain studies that investigate the predictive feature of low-level maternal serum pregnancy-associated plasma protein A (PAPP-A) and high-level serum-free beta hCG (fβ-hCG) values in first trimester screening test [2-8]. In these studies, predictive feature of the values has been investigated for pregnancy complications such as hypertensive disease in pregnancy, preeclampsia, intrauterine growth retardation (IUGR), small fetus for gestational age (SGA) or large fetus for gestational age (LGA), low birth weight (LBW), macrosomia, preterm labor, abortus, preeclampsia and ectopic pregnancy, except for chromosome anomaly.

Metalloproteinase domain-containing protein 12 (ADAM12) is a multidomain glycoprotein belongs to ADAM metalloproteinase family originated from placenta and acting important roles in fetal and placental growth and development [9, 10]. Human ADAM12 enzyme is made of two forms as ADAM12-L (long) and ADAM12-S (short). ADAM12-S, known as secreted form is an insulin-like growth factor-binding protein (IGFBP) protease that splits IGFBP 3 and 5 specifically, thus enhancing the effect of IGF [11-13]. Starting to increase from 8th to 16th week of pregnancy, serum concentration of ADAM12-S increases 60 times during normal pregnancy and reaches to its highest level at term [14, 15]. Laigaard *et al.* [15] stated that serum concentration of ADAM12-S was observed in low levels in pregnancies with trisomy 21.

Measuring the amount of serum ADAM12 has been suggested as an adjunct to screening tests in order to increase the diagnostic efficiency of various fetal diseases, pregnancy complications, and abnormal placental growth in first and second trimester pregnant women [9, 16-22]. When ADAM-12 has been used together with first trimester screening tests, including age of mother + serum β-hCG + PAPP-A + nuchal translucence (NT), detection rate of Down Syndrome (DS) was notified as 97% with a five per-

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cent false positivity [15].

This study is the first in Turkey to investigate the levels of ADAM-12 in first trimester pregnant women. In the study, relationship between combined test components including fβ-hCG, PAPP-A, and NT used in 11-14th weeks of pregnancy for DS screening in first trimester pregnant women and the levels of ADAM12, which is suggested as a new identifier, has been investigated. Also, the authors aimed to determine whether these tests could predict adverse complications of pregnancy such as LBW which could develop in further weeks of pregnancy.

Materials and Methods

Ethical consent of the study was received from the Ministry of Health Clinical Research Ethics Committee of Istanbul Training and Research Hospital. NT and ultrasonography crown-rump length (CRL) measurements of the fetuses were performed in Gynecological Diseases and Obstetrics Polyclinic by physician specialists who had ultrasonography certificates of competence in prenatal screening.

Five ml blood was taken from pregnant women and placed in a straight tube that was centrifuged in 1,500 g for 15 minutes. The serum levels of PAPP-A and fβ-hCG were measured using chemiluminescence method auto analyzer. To determine the week of pregnancy, CRL measurements on the day of taking serum sample were based on.

fβ-hCG and PAPP-A values of pregnant women; NT and CRL measurement results of the fetuses along with the dates of the measurements; the ages, weights, smoking status, diabetes status, ethnic origins, the number of fetuses, whether or not in vitro fertilization of pregnant women, and sample-taking dates were entered into PRISCA 4.0 (Prenatal Risk Measurement, Typolog Software) program. The corrected multiple of median (MoM) value for every pregnant woman was calculated and personal risks for every pregnant woman were determined by using Prisca program.

For the risk group, 40 pregnant women was selected according to biochemical trisomy 21 risk and/or combined trisomy 21 risk > 1:250. Thirty-nine pregnant women with biochemical trisomy 21 risk and/or combined trisomy 21 risk < 1:10000 were selected for the control group. The samples of the pregnant women recruited to the study were stored for maximum six months at -20°C and -40°C without any refreeze or unfreeze until the day of analysis.

The levels of serum ADAM-12 were measured using CA-2000 micro-plate reader device and quantikine human ADAM12 immune analyze ELISA kit.

The information about whether amniocentesis performed and its results; birth time of the pregnant women and, birth weights of the babies was obtained by telephone interview. The other information about pregnant women was obtained from prenatal screening form and double screening test result reports.

The pregnant women were divided into two groups as risk and control were then divided into subgroups according to their baby birth weights: LBW, NBW, and macrosomia (the babies whose birth weights were under 2,500 grams, between 2,500-3,999 grams, and above 4,000 grams were considered as LBW, NBW, and macrosomia babies, respectively).

In the study, SPSS 17.0.1 Statistical Package was used for statistical analysis. The compliance of continuous variables in the study to normal distribution were investigated using Kolmogorov-Smirnov test. The variables indicating Gaussian distribution were shown as minimum-maximum values and mean ± SD values while the values indicating non-Gaussian distribution were shown as minimum-maximum values and median (25.–75. percentile) values.

In comparison of continuous measurements between independent groups, Student's *t*-test was used when Gaussian distribution assumptions were obtained. Where the assumptions were not provided or the sample size n < 30, Mann-Whitney U test was used for comparison.

Correlations between variables were analyzed by Pearson's correlation coefficient (*r*) where the Gaussian distribution assumptions were obtained. Where the assumptions were not fulfilled, Spearman correlation coefficient (r_S) was used.

Statistical significance level was taken as confidence level of 95% and $p < 0.05$ for all the tests.

Table 1. — *Comparisons of the results of risky and control pregnant women.*

Variables	Risky group (n=40)	Control group (n=39)	*p*
Maternal age (years)	18.7-33.8 26.458±4.088	18.4-31.1 25.544±3.146	0.270
Maternal weight (kg)	40-82 60.68±8.589	46-80 61.37±8.194	0.712
Week of pregnancy at first trimester screening test (day)	78-95 87.23±4.446	77-95 87.44±4.547	0.835
CRL (mm)	45.6-78.4 62.305±8.584	45.4-78 62.449±8.782	0.942
Free β-hCG (MoM)	0.63-8.09 2.574±1.616	0.44-1.23 0.791±0.209	<0.000*
PAPP-A (mlU/mL)	0.337-3.87 1.454±0.778	1.32-8.93 4.336±1.587	<0.000*
PAPP-A (MoM)	0.14-0.74 0.424±0.161	0.87-1.56 1.237±0.193	<0.000*
ADAM-12 (ng/mL)	7.415-31.372 17.579±6.217	12.213-51.73 28.807±9.333	<0.000*
NT (mm)	0.8-3.4 1.4(1.2-1.775)	0.7-2.2 1.3(1.1-1.8)	0.222
NT (MoM)	0.62-2.28 0.89(0.792-1.095)	0.54-1.23 0.84(0.76-1.05)	0.266
Free β-hCG (ng/mL)	26.5-415 79(50.17-135.25)	17.2-57.6 28.7(22.9-38.2)	<0.000*
Duration of pregnancy (days)	224-287 273(266-280)	245-294 273(266-280)	0.795
Baby birth weight (grams)	1600-4180 3230(2800-3497.5)	2420-4970 3425(3140-3600)	0.029**

*$p < 0.01$, **$p < 0.05$.

Results

The variables and statistical analyzes of pregnant groups included in the study are shown in Table 1. A statistically significant difference was found between risky and control groups, between variable means or medians of fβ-hCG MoM, PAPP-A, MoM and ADAM-12 (p <0.01), and baby

Table 2. — *Data comparison of macrosomia and NBW baby groups.*

Variables	Macrosomia group (n= 8) min-max Median (25.-75. percentile)	NBW group (n=63) min-max Median (25.-75. percentile)	*p*
Maternal age (years)	24.7-29.5 25.3(25.4-29.25)	18.4-33.8 25.3(23-29.1)	0.300
Maternal weight (kg)	54-70 63.5(58-67.75)	46-82 60(55-68)	0.455
Week of pregnancy at first trimester screening test (days)	85-95 90(86.25-93)	77-95 87(85-90)	0.108
CRL (mm)	57.8-78.0 67.8(59.825-75.1)	45.4-77.8 61.6(55.5-68.1)	0.082
NT (mm)	1.1-1.8 1.35(1.225-1.675)	0.7-2.2 1.4(1.2-1.8)	0.993
NT (MoM)	0.76-1.1 0.87(0.762-1.032)	0.54-1.57 0.86(0.77-1.05)	0.891
Free β-hCG (ng/mL)	99.6-81.9 34.7(21.925-47.0)	17.2-415 42.9(26.0-78.2)	0.244
Free β-hCG (MoM)	0.45-2.92 0.985(0.69-1.327)	0.44-8.09 1.0(0.74-2.09)	0.495
PAPP-A (mlU/mL)	0.976-5.710 3.365(1.095-4.987)	0.337-8.93 2.38(1.45-4.55)	0.636
PAPP-A (MoM)	0.22-1.52 0.98(0.275-1.317)	0.14-1.56 0.94(0.42-1.24)	0.764
ADAM-12 (ng/mL)	10.602-50.315 23.04(16.30-33.12)	8.381-51.73 24.10(15.85-28.74)	0.827
Duration of pregnancy (days)	266-283 273(267.75-280)	252-294 273(266-280)	0.911
Baby birth weight (grams)	40000-4970 4240(4070-4446.25)	2660-3920 3300(3110-3500)	<0.000*

**p < 0.01*
NBW: normal birth weight.

Table 3. — *The data comparison of LBW group to macrosomia baby group.*

Variables	LBW group (n= 8) min-max median (25.-75. percentile)	NBW group (n=63) min-max median (25.-75. percentile)	*p*
Maternal age (years)	21.5-31.6 24.6(22.675-27.9)	18.4-33.8 25.3(23-29.1)	0.707
Maternal Weight (kg)	40-76 61.5(47-68.75)	46-82 60(55-68)	0.880
Week of pregnancy at first trimester screening test (days)	78-95 85(83.5-92.25)	77-95 87(85-90)	0.791
CRL (mm)	46.4-78.4 57.2(55.725-73.2)	45.4-77.8 61.6(55.5-68.1)	0.916
NT (mm)	0.8-3.4 1.4(1.175-2.27)	0.7-2.2 1.4(1.2-1.8)	0.473
NT (MoM)	0.65-2.28 0.95(0.755-1.307)	0.54-1.57 0.86(0.77-1.05)	0.377
Free β-hCG (ng/mL)	34.9-314 92.6(44.0-156.25)	17.2-415 42.9(26.0-78.2)	0.031**
Free β-hCG (MoM)	0.84-5.47 2.65(1.07-4.047)	0.44-8.09 1.0(0.74-2.09)	0.029**
PAPP-A (mlU/mL)	0.46-3.87 1.9(1.125-2.78)	0.337-8.93 2.38(1.45-4.55)	0.281
PAPP-A (MoM)	0.25-0.97 0.475(0.435-0.607)	0.14-1.56 0.94(0.42-1.24)	0.133
ADAM-12 (ng/mL)	7.415-22.562 16.33(13.18-19.90)	8.381-51.73 24.10(15.85-28.74)	0.033**
Duration of pregnancy (days)	224-283 262(245-273.75)	252-294 273(266-280)	0.024**
Baby birth weight (grams)	1600-2500 2280(2012.5-2465)	2660-3920 3300(3110-3500)	<0.000*

LBW: low birth weight
NBW: normal birth weight
**p < 0.01. **p < 0.05.*

birth weights ($p < 0.05$).

The data comparison of NBW group and LBW group to macrosomia baby group is shown in Tables 2 and 3.

When comparing LBW and NBW groups, a statistical difference was found between medians of variables of baby birth weight, fβ-hCG MoM, ADAM12, and pregnancy duration ($p < 0.001$; $p = 0.029$; $p = 0.033$, and $p = 0.024$, respectively). A statistically significant difference was found between macrosomia baby group and NBW group with respect to medians of variables for the birth weights of the babies ($p < 0.001$).

Correlation coefficients and *p* values of these coefficients between ADAM12 levels and other data of pregnant women are shown in Table 4. A statistically significant correlation was determined between ADAM12 and PAPP-A MoM variables of all pregnant women included in the study ($r = 0.635$, $p < 0.001$).

Six of 40 pregnant women (15%) identified as having risk according to double screening test were found to have amniocentesis as an advanced diagnostic procedure and only one of them (2.5%) had a DS (trisomy 21) baby. Since pregnant women having DS baby was not sufficient in number, statistical analysis could not been performed.

Discussion

In the present study, the levels of ADAM12 in risk group including one pregnant woman with DS baby were significantly low (statistically) in comparison to control group. This is consistent with the finding of Laigaard *et al.* [14, 15] who reported that the concentration of ADAM12 was significantly lower in pregnancies with trisomy 21.

Wortelboer *et al.* [21] reported ADAM12 MoM values in patients with 218 DS, 62 trisomy 18 (Edwards syndrome), and 29 trisomy 13 cases. They stated that these values were low in early periods of first trimester for all trisomy cases but adding ADAM 12 did not enhance the screening performance. They also suggested that ADAM 12 could be used as an additional biochemical indicator in first trimester screening test for trisomy cases apart from DS.

Table 4. — *Correlation coefficients and p values of these coefficients between ADAM12 levels and other data of pregnant women*.

Variables	ADAM12 (ng/mL)					
	Risky group (n=40)		Control group (n=39)		All pregnants (n=79)	
	r	*p*	*r*	*p*	*r*	*p*
Maternal age (years)	0.062	0.703	-0.143	0.386	-0.109	0.341
Maternal weight (kg)	-0.378	0.016*	-0.414	0.009**	-0.291	0.009*
Week of pregnancy at first trimester screening test (days)	0.330	0.038*	0.099	0.549	0.167	0.142
CRL (mm)	0.299	0.061	0.067	0.686	0.133	0.244
NT (mm)	0.138	0.396	-0.065	0.695	-0.057	0.621
NT (MoM)	0.033	0.840	-0.061	0.713	-0.110	0.334
Free β-hCG (ng/mL)	0.183	0.258	-0.091	0.580	-0.256	0.023*
Free β-hCG (MoM)	0.281	0.079	-0.147	0.372	-0.269	0.017*
PAPP-A (mlU/mL)	0.380	0.016*	0.349	0.030*	0.630	<0.000**
PAPP-A (MoM)	0.186	0.250	0.378	0.018*	0.635	<0.000**
Duration of pregnancy (days)	-0.055	0.736	-0.021	0.897	0.025	0.828
Baby birth weight (grams)	0.105	0.519	0.077	0.643	0.236	0.036*

*r: Pearson correlation, **p < 0.01, *p < 0.05.*

In the present study, a significant difference was not found between the level of ADAM12 (28.077 ng/mL) at 13th week pregnant woman having a fetus with DS and the control group average (28.807 ng/mL). The reason for this is that as suggested by Laigaard *et al.* [15] and Wortelboer *et al.* [21], although the difference between DS affected pregnancies and those not affected with regards to the levels of ADAM12 is at its peak in the 8th week of pregnancy, the difference decreases progressively during pregnancy. The present findings seems to support the consideration [14] that ADAM12 could be highly useful in distinguishing trisomy 21 pregnancies only before the 10th week of pregnancy.

In the present study ADAM12 levels were also significantly low in pregnant women with a LBW baby with respect to those with NBW baby. These findings are consistent with the findings of Laigaard *et al.* [15], Canick [7] and, Cowans and Spencer [9]. From this point of view, as a result of dissociated IGFs from IGFBPs to induce cellular growth, increased ADAM12 levels may be suggested to cause an increase in fetal growth and development.

In consistent with the findings of Cowans and Spencer [9] and Laigaard et al. [17], in our study, ADAM12 levels were found to correlate positively with the levels of PAPP-A in all the pregnant women included in the study.

In our study, fβ-hCG levels in risk group including DS case were found significantly high statistically. Our finding is consistent with the results of Bogart [23] and Macri [24] et al. who reported high hCG and β-hCG in pregnant women with trisomy 21 baby. Besides, MoM value of fβ-hCG of pregnant woman with DS baby is 5.15 in our study. This value was found to be very high when comparing risk group and control group and fβ-hCG MoM values of control group.

Serum β-hCG MoM values of the group with LBW baby were found significantly high in comparing to the group with normal birth weight baby. Our finding is inconsistent with the findings of Dugoff *et al.* [5] and Krantz *et al.* [25] who reported that low fβ-hCG levels were seen along with LBW.

In their studies, Canick *et al.* [7] and Spencer *et al.* [26], reported that low PAPP-A MoM values were found together with genetic anomalies. Therefore, these pregnant women should be regarded at high risk. Also, Marttala *et al.*, [26] reported that using low PAPP-A levels in order to determine and prevent the complications of unwanted pregnancies earlier is important in identifying high-risk group. The present finding that low PAPP-A levels determined in risk group is consistent with the findings of Canick *et al.* [7], and Spencer *et al.* [26], and Marttala *et al.* [27].

A significant positive correlation was identified between first trimester relative birth weights and PAPP-A values, as well as birth weight and maternal serum PAPP-A levels in late periods of pregnancy [28, 29]. In the present study, a statistically significant correlation was not found between PAPP-A MoM values of the pregnant women and baby birth weights. This finding is consistent with the results of Dugoff *et al.* [5], whereas inconsistent with those of Pedersen *et al.* [28], Bischof *et al.* [29], and Peterson *et al.* [30]. In the present study, another prominent and coherent information with the findings is that, although PAPP-A value (0.18 MoM) of pregnant woman having a fetus with DS was considerably low compared to mean PAPP-A MoM values (1.2 MoM) of healthy pregnant women in control group, DS baby was born at term and NBW.

Birth weight is an important factor in determining peri-

natal morbidity and mortality. Birth asphyxia, meconium aspiration, pulmonary hemorrhage, hyperviscosity syndrome, and hypoglycemia could be seen along with IUGR [23]. On the other hand, birth weight depends on gestational age and fetal development. In the present study, baby birth weights of risk group were found to be significantly low in compared to control group. Observing low birth weight along with low levels of ADAM12 and PAPP-A in risk group indicates that these variables could have similar effects on baby birth weights.

Although low levels of ADAM12 is regarded to be related to pregnancies with negative results, measured ADAM12 concentrations vary considerably among studies. The present authors agree with the opinion of Cowans *et al.* [31] who suggested that these differences in ADAM12 levels may result from the differences in measurement method of ADAM12, the type of antibody used, the week of pregnancy when testing, correction factors, maternal factors, and stabilities of the samples. As a result, low levels of serum ADAM-12 determined in first trimester pregnant women could be used as a new bioindicator supporting other parameter's diagnostic effectivity in prenatal screening and identifying pregnancies with DS risk. The studies of first-trimester ADAM12 levels are very rare in the world and the present study is the first research in this country. The limitation of this study is the relatively low number of samples. A real participation of ADAM12 in prenatal screening is considered only possible through the studies which include more fetuses with genetic abnormalities in sample groups and investigate the pregnancies with defects concerning pregnancy.

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Corresponding Author:
S. YILDIRMAK, M.D., PhD
Giresun Universitesi Tip Fakultesi Gazipasa
Yerleskesi Dekanlik Binası
Merkez-Giresun (Turkey)
e-mail: yildirmaksembol@gmail.com