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First trimester biochemical markers in twin pregnancies

Biochemiczne markery pierwszego trymestru w ciąży bliźniaczej

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Abstract

Objective: *Our aim was to investigate the first trimester serum markers and nuchal translucency (NT) measurements in twin pregnancies in our population.*

Materials and methods: *We reviewed the results of all double tests that were performed in our hospital over a three-year period. Out of them, we selected all twins and compared them with a group of three times as many singleton controls. NT measurements and the first trimester serum markers from 49 twin pregnancies were compared to those of 147 pregnant women with normal singleton pregnancy.*

Results: *There were no statistically significant differences in age, gestational age and maternal weight between the two groups (p>0.05). We found similar NT measurements in the two groups. The median MoM of Pregnancy-Associated Plasma Protein A (PAPP-A) and fβ-hCG levels in twins were statistically significantly higher than those in singleton pregnancies. Twelve percent of the twins (12.2 %) were the result of assisted reproduction technologies. IVF versus naturally conceived pregnancies showed similar MoM of PAPP-A (2.2 vs. 1.2, respectively) and fβ-hCG (Mann-Whitney U; p = 0.195 and p = 0.958).*

Conclusions: *Our study revealed that median PAPP-A and fβ-hCG levels for twins were less than twice those of singleton values.*

Key words: **nuchal translucency** / **pregnancy-associated plasma protein A** / / **twins** / **screening** /

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Streszczenie

Cel pracy: *Celem naszego badania było zbadanie surowiczych markerów pierwszego trymestru oraz przezierności karkowej (NT) w ciążach bliźniaczych w naszej populacji.*

Metoda: *Przeanalizowano wyniki wszystkich testów podwójnych wykonanych w naszym szpitalu w ciągu trzech lat. Wyodrębniono grupę ciąż bliźniaczych (n=49) i porównano ją z trzy razy większą grupą kontrolną prawidłowych ciąż pojedynczych (n=147).*

Wyniki: *Nie znaleziono istotnych statystycznie różnic w wieku, wieku ciążowym i masie ciała ciężarnych pomiędzy grupami (p>0,05). Stwierdzono podobne wyniki pomiaru NT w obu badanych grupach. Mediana MoM białka PAPP-A i poziom fβ-hCGw ciążach bliźniaczych był istotnie wyższy niż w ciążach pojedynczych. Dwanaście procent ciąż bliźniaczych było efektem technik wspomaganego rozrodu. Ciąże w wyniku IVF oraz ciąże spontaniczne wykazywały się podobnych wynikiem MoM białka PAPP-A (2,2 vs. 1,2 odpowiednio) i fβ-hCG (Mann-Whitney U; p=0,195 i p=0,958).*

Wnioski: *Średni poziom białka PAPP-A i fβ-hCG dla ciąż bliźniaczych był mniej niż dwa razy większy niż dla pojedynczych ciąż.*

Słowa kluczowe: **przezierność karkowa** / **białko PAPP-A** / **bliźnięta** / **skrining** /

Introduction

The prevalence of Down syndrome (DS) in twins (1/649) is similar to that in singletons (1/754) [1-3]. Effective risk assessment for fetal aneuploidy is important in twin pregnancies.

The first trimester combined screen test measures maternal serum levels of free beta- hCG and pregnancy-associated plasma protein-A (PAPP-A) at 9–12 weeks gestation and measures nuchal translucency (NT) by ultrasound at 11–13 weeks gestation. Maternal serum screening is limited in twin pregnancies because of lower reported detection rates compared with singleton pregnancies. Serum marker levels in unaffected twin pregnancies are considered to be double those observed in singleton pregnancies [4-7]. NT is obviously an effective marker for aneuploidy risk assessment in twin pregnancies. The sensitivity of NT measurements in twins is similar to singletons.

Our objective was to investigate the maternal serum concentrations of first trimester serum markers and NT measurements in twins and compare with singletons in our population.

Material and methods

We reviewed the results of all double tests that were performed in our hospital in a three-year period. Out of them, we selected all twins and compared them with a group of three times as many singleton controls. Patients who were included in the study group had a viable twin pregnancy and underwent first-trimester screening with maternal age, NT, PAPP-A, and free beta-hCG. After each twin pregnancy, the following three normal pregnancies were selected as controls. Pregnancies were recruited consecutively. All singletons were matched one-to-one with those of age-matched twins. The controls were selected from the same database. In our clinic, all blood samples were accompanied by a form with information relevant for the risk calculation (e.g. date of birth, gestational age, maternal weight, insulin dependent diabetes mellitus status, number of fetuses, smoking status, NT). This protocol was approved by the Research Ethics Committee of our hospital.

NT measurements and the first trimester serum markers from 49 twin pregnancies were compared to those of 147 pregnant women with normal singleton pregnancy receiving routine antenatal care in our clinic. Maternal age, gestational age, maternal weight, maternal smoking status, NT and crown–rump length (CRL) were recorded. Gestational age was determined by first-trimester ultrasonography (CRL) in the vast majority of cases. When unknown, the date of self-reported last menstrual period or gestational age known by the patient was taken into account.

Serum samples were drawn between 11 and 13 weeks gestation. First-trimester screening was performed after informed consent at 11–13^{6} weeks of pregnancy with a CRL of 45–80 mm. The Down's syndrome screening program in our laboratory includes free beta- hCG and PAPP-A. Levels of PAPP-A, free beta-hCG were measured with the Immulite 2000 Analyzer (EURO/DPC Ltd). Screening for Down's syndrome is performed once a week (~80 samples each run). Correction was performed for weight and smoking. In cases of twins, we used the correction factors published by Wald [8]. Concentrations of the serum markers were expressed in multiples of the medians (MoM) for pregnancies of the same gestational age. All markers were expressed as multiples of the normal median for women with unaffected pregnancies at a given gestational age. Fetal crown–rump length (CRL) and fetal NT were measured by transabdominal ultrasound examination. NT was measured using the maximum vertical distance between the skin and subcutaneous tissues at the back of the neck in a sagittal section of the fetus lying in the neutral position, by sonographers who received Certificate of Competence in first-trimester scanning. NT was expressed in MoMs.

Statistical analyses were performed using SPSS version 15.0 (SPSS Software, Chicago, IL). All data sets were subjected to normality testing using the Kolmogorov–Smirnov method. The data were reported as mean ± standard deviation (for normally distributed data) or as median and range (for non-normally distributed data). Comparisons between two groups were performed using Student's *t*-tests or Mann–Whitney rank sum tests. *P*-values of < 0.05 were considered statistically significant.

Results

During the study period 36378 patients had first trimester screening in the unit. Serum first trimester serum markers were determined in a case-control study of 49 twins and 147 singletons. The baseline characteristics of the twins and singletons are shown in Table I. There were no statistically significant differences in age, gestational age, maternal weight and nuchal translucency between the two groups (p>0.05). The median MoM of PAPP-A in twins was significantly higher than that in singleton pregnancies (p=0.0001). Serum fβ-hCG levels in twins were also significantly higher than singletons (p=0.0001). Median PAPP-A and hCG levels for twins were less than twice those of singleton values. We also found similar NT measurements in the two groups.

Twelve percent of the twins (12.2%) were the result of assisted reproduction technologies. IVF versus naturally conceived pregnancies showed similar MoM of PAPP-A (2.2 vs. 1.2, respectively) and fβ-hCG (Mann-Whitney U; p = 0.195 and p = 0.958).

There was no case of trisomy 21 in twins and singletons in the study period.

Discussion

The rate of multiple pregnancies is increasing due to advancing maternal age and greater use of assisted reproduction techniques. Measurement of maternal serum markers is a well established screening test for Down syndrome. However, the use of such screening in multiple pregnancies remains controversial because of lower reported detection rates compared with singleton pregnancies [9].

In our study, serum first trimester serum markers were determined in only 49 twins because in our clinic the Down syndrome screening program did not officially include serum screening for twin pregnancies and screening was only performed on specific request. Currently, risk calculations in twins are based on NT measurements of each individual fetus.

Koster et al. [10], revealed that median MoMs of PAPP-A and f*β*-hCG were approximately twice as high in twins as compared to singleton pregnancies. Table 2 demonstrates first trimester median maternal serum screening marker levels (MoM) in twins from different studies [11-14]. Our study revealed that median PAPP-A and hCG levels for twins were less than twice those of singleton values. Our PAPP- A levels were comparable to medians found in the study of Gonce et al., [14].

We found similar NT measurements in the two groups. The sensitivity of NT measurements in twins is similar to singletons. Sepulveda et al. [15], have confirmed that first-trimester NT thickness measurement is a highly sensitive technique for detecting chromosomally abnormal fetus in multiple pregnancies.

No statistically significant difference was found in PAPP-A or f*β*-hCG MoMs between IVF versus naturally conceived pregnancies, which was different from the study reported by Amor et al., [16]. Similarly, Geipel et al., revealed that first-trimester maternal serum free β-human chorionic gonadotropin and PAPP-A levels were more obviously altered in assisted conception [17].

Table I. Demographic and laboratory characteristics in two groups.

	Twins (n=49)	Singletons (n=147)	P
Maternal age (years)*	30.1 ± 4.5	29 ± 5.2	0.165
Gestational age (days)**	85 (77-97)	85 (75-95)	0.342
Maternal weight (kg)**	63 (46-77)	61 (43-95)	0.844
PAPP-A (MoM) **	1.4 (0.2-4.4)	0.8 (0.07-4.1)	0.0001
HCG (MoM) ** NT (MoM)**	1.6 (0.36-4.1) 0.89 (0.35-1.74)	0.9 (0.22-7.4) 0.97 (0.39-2.2)	0.0001 0.178

Abbreviations:
HCG: human chorionic gonadotropin, MoM: Multiples of the medians, NT: Nuchal translucency,
PAPP-A: Pregnancy-associated plasma protein-A,
* Values are mean ± SD
**Values are median (minimum-maximum)

Table II. First trimester median maternal serum screening marker levels (MoM) in twins.

	Twins (n)	fβ-hCG	PAPP-A
Spencer, 2000 (11)	159	2.10	1.86
Niemimaa, 2002 (4)	67	1.85	2.36
Orlandi, 2002 (12)	30	1.72	1.61
Bersinger, 2003 (13)	68		1.87
Mashiach, 2004 (6)	93	2.18	2.38
Gonce, 2005 (14)	98	1.57	1.96
Wojdemann, 2006 (7)	128	2.06	2.14
Present study, 2014	49	1.6	1.4

As a conclusion, median PAPP-A and hCG levels for twins were less than twice those of singleton values. Larger studies including twins with Down syndrome are necessary to determine the optimal method of screening for aneuploidy in twins.

Conflict of interest: No conflict of interest was declared by the authors.

Authors' contribution:

1. Şevki Çelen – concept, study design, analysis and interpretation of data.
2. Yaprak Engin-Üstün – concept, assumptions, study design, article draft, analysis and interpretation of data, corresponding author.
3. Figen Türkçapar – acquisition of data, analysis.
4. Ayla Aktulay – acquisition of data, analysis.
5. Nafiye Yılmaz – revised article critically.
6. Ayeşegül Öksüzoğlu – acquisition of data, analysis.
7. Özlem Yörük – acquisition of data, analysis.
8. Nuri Danışman – revised article critically.

Authors' statement

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