J Med Biochem 2011; 30 (2) DOI: 10.2478/v10011-011-0010-x

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 30: 126–130, 2011

Original paper
Originalni naučni rad

FALSE POSITIVE VALUES OF BIOMARKERS OF PRENATAL SCREENING ON CHROMOSOMOPATHY AS INDICATORS OF A RISKY PREGNANCY

LAŽNO POZITIVNE VREDNOSTI BIOMARKERA PRENATALNOG SKRININGA NA HROMOZOMOPATIJE KAO POKAZATELJI RIZIČNE TRUDNOĆE

Jasmina Durković[1], Luka Anđelić[2], Bojana Mandić[3], Denis Lazar[3]

[1]Department of Genetics, Hospital Subotica
[2]Department of Obstetrics and Gynecology, Hospital Subotica
[3]MegaLab Biochemical Laboratory Subotica, Subotica, Serbia

Summary: Genetic screening on chromosomopathy has been performed on 2000 pregnant women in their first trimester of pregnancy by determining Pregnancy associated plasma protein-A and free-beta HCG biomarkers in maternal serum. After obtaining a normal fetal karyotype, the pathological values of the biomarkers have been correlated with other pregnancy disorders, and the possible causes of the positive genetic screening have been tested. 340 false positive biomarkers (17%) have been detected. The increased free-beta HCG (48.24%) had a significant influence. A significant correlation ($p > 0.01$) between the increased free-beta HCG and bleeding during pregnancy has been established. Complications occurred in 78.52% pregnancies with pathological biomarkers, MISSed in 13.82%, miscarriages in 10.88%, induced pregnancy terminations caused by fetal anomalies in 8.82% and births with disturbed fetal vitality in 45%. The research results have shown a significant correlation ($p > 0.01$) between the increased value of the free-beta HCG biomarkers and fetal hypoxia. The false positive genetic screening, caused by the increased free-beta HCG, can indicate placental dysfunction and fetal vitality disruption.

Keywords: prenatal screening, chromosomopathy, risky pregnancy, false positive values

Kratak sadržaj: Kod 2000 trudnica urađen je u prvom trimestru trudnoće genetski skrining na hromozomopatije određivanjem biomarkera Pregnancy associated plasma protein-A i free-beta-HCG u maternalnom serumu. Posle dobijanja normalnog kariotipa fetusa, patološke vrednosti biomarkera su korelisane sa drugim poremećajima trudnoće kako bi se ispitali mogući uzroci pozitivnog genetskog skrininga. Otkriveno je ukupno 340 lažno pozitivnih nalaza biomarkera (17%). Značajan udeo imao je povišeni free-beta-HCG (48,24%). Utvrđena je značajna povezanost ($p>0,01$) povišenog free-beta-HCG i krvarenja u trudnoći. U 78,52% trudnoća sa patološkim biomarkerima nastale su komplikacije: 13,82% MISSed, 10,88% spontani pobačaj, 8,82% indukovani prekid trudnoće zbog anomalija ploda i 45% porođaja sa poremećajem fetalnog vitaliteta. Rezultati istraživanja su pokazali veoma značajnu povezanost ($p>0,01$) između povećane vrednosti biomarkera free-beta-HCG i fetalne hipoksije. Lažno pozitivan genetski skrining uzrokovan povišenim free-beta-HCG može da bude pokazatelj placentarne disfunkcije i poremećaja fetalnog vitaliteta.

Ključne reči: prenatalni skrining, hromozomopatije, rizična trudnoća, lažno pozitivne vrednosti

Introduction

Genetic screening on chromosomopathy is performed in the first trimester of pregnancy, between the 11th and 14th week, by determining the nuchal translucency of a fetus (of the cervical crease – NT) with the ultrasound and the fetoplacental Pregnancy associated plasma protein-A (PAPP-A) and free human chorionic gonadotropin (free-beta HCG) biomarkers in maternal serum. PAPP-A is a glycoprotein synthesized in the trophoblastic placental tissue. In

Address for correspondence:

Prim dr sc. med Jasmina Durković
Department of Genetics
Town Hospital Subotica, Serbia
Izvorska 3
Tel: 024 555 222 ext. 404, Fax 024 555 267
Mobile phone: 063 224 324
e-mail: jasminadurkovic@gmail.com

trisomy 21, 18 and 13, concentrations in maternal serum are more than 40% lower than in normal pregnancy. Free-beta HCG is a hormone which produces syncytiotrophoblast. Its increased level in pregnancy is considered the most sensitive marker in detecting trisomy 21, while in trisomy 18 and 13 the values are much lower. The value of each biomarker is calculated in conventional units and in relation to a median (MOM – multiple of median). The sensitivity of genetic screening has been improved with the use of software which adapts the values of fetal biomarkers in the serum according to the mother's age, weight, ethnic group, diabetic status, the number of smoked cigarettes and gestational age calculated with the ultrasound by measuring the crown-rump length (CRL). By combining the biomarkers with the ultrasound marker NT, an individual risk expressed as 1:n can be obtained. As a cut-off risk indicating prenatal karyotyping, 1:270 is used which corresponds to a pregnant woman aged 35.

It has been estimated that the rate of false positive values of genetic screening is about 5%, which has resulted in the increased number of invasive diagnostic procedures of prenatal karyotyping in risk free pregnant women with respect to age, but also in the negative implications on the psychological condition of a pregnant woman (12–14). The causes of the false positive results of genetic screening have lately been increasingly investigated and the model for the reading of fetal biomarkers as well as for the interpretation of pathological values has been sought for.

Through the prospective and partly retrospective analysis of genetic screening on chromosomopathy in the first trimester of pregnancy, the frequency of false positive results has been tested. In normal fetal karyotyping, the correlation between the pathological values of biomarkers and other pregnancy disorders has been sought out. Is false genetic screening worthless after the prenatal karyotyping and normal fetal karyotyping? Are the pathological values of the biomarkers indicators of other possible risks in pregnancy?

Material and Methods

2000 pregnant women have been tested. In the first trimester of their pregnancy the genetic screening on chromosomopathy has been performed, the nuchal translucency of the fetus (NT) has been measured, and the PAPP-A and free-beta HCG biomarkers in maternal serum have been determined. From every pregnant woman a detailed personal, gynecological, hereditary and teratogenic anamnesis has been taken, while monitoring their respective health status during pregnancy, the outcome of the pregnancy and the health status of the neonate. PAPP-A and free-beta HCG biomarkers have been read with IMMULITE 2000 SIEMENS which operates on the principle of chemiluminescence, using the original reagents (15). The processing of data and determination of the risk of trisomy 21 and 18 have been done with PRISCA 4 SOFTWARE (16).

For the statistical processing of the data in a dichotomous manner, i.e. to establish whether a marker is normal or pathological, whether there is a risk or not, the table of contingency (chi-quadrant test) has been used.

Results

Out of 2000 pregnant women tested, there were 138 (6.9%) false positive screening results with the final risk in PRISCA software higher than 1:250 indicating prenatal karyotyping, while 202 pregnant women (10.1%) had a final risk higher than the initial age risk. 340 pregnant women with bad genetic screening results were submitted to the fetal karyotyping test and a normal karyotype of the fetus was obtained. In pregnancies terminated in miscarriages the cytogenetic analysis of the miscarried sample of the chorion and fetal tissue has been performed and a normal karyotype has been obtained which proved the false positive result of genetic screening.

Only 5.3% of the false positive results had a pathological NT marker, while in 94.7% false results were caused by the pathological values of PAPP-A and free-beta HCG biomarkers. Increased free-beta HCG had the largest influence on false positive results (48.24%).

The connection between the increased value of free-beta HCG over 2 MOM and the pregnancies accompanied by bleeding and maintained by progesterone drugs has been examined. There is a significant connection between the increased value of free-beta HCG over 2 MOM and the maintained pregnancy (*Table I*).

The outcome of pregnancies with false positive genetic screening has been monitored. 33.53% of pregnancies terminated in a miscarriage in the second trimester (*Table II*).

The vitality of a newborn has been examined in 66.47% pregnancies that ended in labour (*Table III*).

Table I The survey of pregnancies with free-beta HCG MOM > 2 compared to maintained pregnancy.

Free beta HCG / Maintained pregnancy	Normal < 2 MOM	Pathological > 2 MOM	Total
No	96	104	200
Yes	80	60	140
Total	176	164	340

$hi^2 = 2.7570$ d.f.=1 p=0.0968

Table II Survey of the outcome of pregnancies with false positive genetic screening.

Pregnancy outcome	Number of pregnant women	Structure %
MISSed	47	13.82%
Miscarriage	37	10.88%
Induced pregnancy termination	30	8.82%
Spontaneous labour	184	54.12%
Caesarean section	38	11.18%
Stillborn	4	1.17%
Total	340	100.00%

Table III Survey of the health status of neonates.

Health status of the neonate	Number of neonates	Structure %
Healthy	73	32.30 %
Diseased	153	67.70 %
Total	226	100.00 %

Table IV Survey of the pregnancies with free-beta HCG > 2 compared to fetal hypoxia.

Free beta HCG / Fetal hypoxia	Normal < 2 MOM	Pathological > 2 MOM	Total
Vital	79	74	153
With hypoxia	47	26	73
Total	126	100	226

$hi^2 = 3.2564$ d.f.=1 p=0.0712

The connection between increased free-beta HCG over 2 MOM and fetal hypoxia has been analysed. A significant connection was established between the increased value of free-beta HCG over 2 MOM and fetal hypoxia (*Table IV*).

Discussion

The free-beta HCG biomarker is the most frequent cause of false positive results of genetic screening with values higher than 2 MOM, thus its correct interpretation and correlation with the pregnancy pathology is necessary.

The increased free-beta HCG over 2 MOM is particularly noticeable in pregnant women who have suffered from threatened miscarriage, bleeding in the early pregnancy and the maintained pregnancy supported by progesterone pills. Of 140 maintained pregnancies with bleeding, the increased free-beta HCG over 2 MOM was noticed in 60 (47.14%). Free-beta HCG is the product of placental syncytiotrophoblast. It is considered that the level of the HCG serum biomarker can also represent a placental marker. Retrospectively, the correlation between beta HCG and placental vascular disruption has been observed, so that the high level of beta HCG markers over 2.5 MOM can indicate a risk of preeclampsia (17, 18). The increased level of free-beta HCG biomarkers is the result of placental trophoblast hyperplasia. In these conditions, the mechanism for the rise of free-beta HCG lies in the focal necrosis of syncytiotrophoblast and the rise of mitotic activity with cellular cytotrophoblast proliferation which is transformed into syncytiotrophoblast. In the transformed syncytiotrophoblast there is an increased production of free-beta HCG.

Out of 340 pregnancies with the false positive genetic screening, 47 (13.82%) ended in MISSed, 37 (10.88%) in miscarriage, 30 (8.82%) by induced pregnancy termination. Among the indicated pregnancy terminations there were 3 cases of anencephaly, 8 of cystic hygroma, 3 of omphalocele, 4 of heart defect, 1 of a diaphragmatic hernia, 3 of Dandy-Walker Syndrome, 2 of polycystic kidney disease, 1 of spina bifida, 3 of multiple anomaly and 2 pregnancies terminated because of teratogenic effects. Fetal karyotyping has been done in all pregnancies before or after the termination and there were no chromosomal aberrations. 226 (66.47%) pregnancies ended in labour, 184 (54.12%) in spontaneous labour, 38 (11.18%) in a Caesarean section and 4 (1.17%) in stillbirth.

Based on the health status of neonates, two groups were formed: healthy and vital babies made up one group, diseased and non-vital babies with normal karyotypes formed the other group. Among the diseased babies were the ones with fetal respiratory distress, asphyxiation, intracranial hemorrhage and convulsions, all resulting from hypoxia. The connection between the level of HCG markers in maternal serum on the one hand and complications in pregnancy on the other has been mentioned in various reference books (19–22). In this research, the condition of fetal hypoxia has been statistically much more often observed in pregnancies with the increased level of HCG markers over 2 MOM than in the control group ($p > 0.01$). Inadequate perfusion leads to reduced placental oxygenation. The condition of hypoxia leads to syncytiotrophoblastic hyperplasia which produces increased HCG and these changes in the placenta are connected to the fatal outcome of pregnancy.

Although the primary risk was low, complications occurred in 78.52% of pregnancies with pathological genetic screening, MISSed in 13.82%, miscarriages in 10.88%, induced pregnancy termination in 8.82% and births with disturbed fetal vitality in 45%.

The results of this study have contributed to the elucidation of the false positive results of genetic

screening and to the determination of the level of risk in a pregnancy. Pathological genetic screening with normal fetal karyotyping can indicate placental dysfunction and fetal vitality disruption. When the performed prenatal cytogenetic analysis has confirmed the fetal karyotype as normal and the genetic screen as falsely positive, it should not be forgotten that the fetoplacental biomarker values were pathological; instead we should look for the cause of their abnormal production. Such pregnancies should be treated as risky and should be clinically and laboratory controlled. In the third trimester there is the possibility of determining other biomarkers indicating fetoplacental vitality, such as human placental lactogen (HPL), glucose tolerance tests, preeclampsia risks, regular ultrasound controls of fetal biometry, estimation of biophysical profile, doppler test of uteroplacental and fetoplacental circulation.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

References

1. Brizot ML, Snijders RJM, Bersinger NA, Kuhn P, Nicolaides KH. Maternal serum pregnancy associated placental protein-A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. Obstet Gynecol 1994; 84: 918–22.
2. Haddow JE, Palomaki GE, Knight GJ et al. Screening of maternal serum for fetal. Down's syndrome in the first trimester. N Engl J Med 1998; 338: 955–61.
3. Powell KJ, Grudzinskas JG. Screening for Down syndrome in the first trimester. Reprod Fertil Dev 1995; 7: 1413–17.
4. De Basio P, Siccardi M, Volpe G. First trimester screening for Down syndrome using nuchal translucency measurement with free-beta HCG and PAPP-A between 10 and 13 weeks of pregnancy – the combined test. Prenat Diagn 1999; 19: 360–3.
5. De Graf IM, Van Bezouw SM, Jacobs ME. First trimester noninvasive prenatal diagnosis of triploidy. Prenat Diagn 1999; 19: 175–7.
6. Spencer K, Sauter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free-beta human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 1999; 13: 231–7.
7. Krantz DA, Hallchan TW, Orlando F, Buchanan P, Larsen JW, Macri JN. First trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. Obstet Gynecol 2000; 96: 207–13.
8. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening of chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-step clinic: a review of three years prospective experience. Br J Obstet Gynaecol 2003; 110 (3): 281–6.
9. Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First Trimester Screening for Trisomies 21 and 18. New Engl J Med 2003; 349: 1405–13.
10. Brigatti KW, Malone FD. First trimester screening for aneuploidy. Obstet Gynecol Clin North Am 2004; 31 (1): 1–20.
11. Wald NJ, Bestwick J, Morris JK. Cross-trimester marker rations in prenatal screening for Down syndrome. Prenat Diagn 2006; 26: 514–23.
12. Wald NJ, Huttly WJ, Rudnicka AR. Prenatal screening for Down syndrome: the problem of recurrent false-positives. Prenat Diagn 2004; 24 (5): 389–92.
13. Summers AM, Huang T, Meier C, Wyatt PR. The Implications of a False Positive Second Trimester Serum Screen for Down Syndrome. Obstet Gynecol 2003; 101: 1301–6.
14. Evers-Kiebooms G, Nys K, Decruyenaere M, Witters I, Fryns JP. Triple Test Screening for Down Syndrome: Looking Back on False-Positive Results and Having or Not Having a Triple test in Subsequent Pregnancies. Community Genetich 2001; 4: 43–9.
15. Bujišić N. Effects of serum-alot confact time on second-trimester prenatal screening markers and their stability on serum. Journal of Medical Biochemistry 2010; 29: 84–88.
16. PRISCA PRENATAL RISK CALCULATION. The screening program under Microsoft Windows. Typolog software. http://www.typolog.de
17. Wald NJ, Morris JK, Ibison J, Wu T, George LM. Screening in early pregnancy for pre-eclampsia using markers from the Down's syndrome Quadruple test. Wolfson Institute of Preventive Medicine. Preliminary results of selected work in progress on Antenatal Screening 2007. MEDLINE.
18. Muller F, Savety L, Le Fiblec B, Bussires L, Ndayizamba G, Claude Colan J, Giraudet P. Maternal serum human chorionic gonadotropin level at fifteen weeks is a predictor for preeclampsia. AMJ Obstet Gynecol 1996; 175: 37–40.
19. Pergament E, Estain AK, Fiddber M, Cho NK, Kupferninc MJ. Adverse pregnancy outcome after a false-positive screening for Down's syndrome using multiple markers. Obstet Gynecol 1995; 13: 58–62.
20. Gonen R, Perz R, David M, Dar H, Merk Samer R, Sharf M. The association between unexplained second trimester maternal serum HCG elevation and pregnancy complications. Obstet Gynecol 1992; 80: 83–6.
21. Van Rijin M, Van der Schouw YT, Hasenaars AM, Visser

GH, Christieans GC. Adverse obstetric outcome in low- and high-risk pregnancies: predictive value of maternal serum screening. Obstet Gynecol 1999; 94 (6): 929– 34.

22. Chandra S, Scott H, Dodds L, Watts C, Blight C, Van Der Hof M. Unexplained elevated maternal serum alpha-fetoprotein and/or human chorionic gonadotropin and risk of adverse outcomes. Am J Obstet Gynecol 2003; 189 (3): 775–81.

Received: October 10, 2010

Accepted: December 1, 2010