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Incidence of pregnancy chromosomal abnormalities detected by screening which include: fetal nuchal translucency thickness, maternal serum free beta human chorionic gonadotrophin hormone, and pregnancy associated plasma protein: A, in Bitola

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Abstract
Introduction: The most effective method of screening chromosomal abnormalities is by a combination of fetal nuchal translucency thickness and maternal serum free beta human chorionic gonadotrophin hormone and pregnancy associated plasma protein-A at the first 10–14 weeks of pregnancy gestation.
Methods: The serum of 526 pregnant women was separated and pregnancy associated plasma protein-A, and free beta human chorionic gonadotrophin hormone were measured. The ultrasound scan included a full structural survey, and nuchal translucency. Risks for chromosomal abnormalities were calculated using the software Prisca - mathematical model which gives individual risks for trisomy 21, 18 and 13.
Results: Over a 2 – year period of time, screening was carried out in 526 pregnancies. Median maternal age was 29,3 years old (range: 13, 4 to 43 years old), and 64 (12, 2%) of women who were 35 years old or older at the time of this assessment. In this prospective study, in among of the 526 pregnant women overall, 48 (9,1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. In the rest of 478 (90, 9 %) cases, chromosomal abnormality was not found.
Discussion: The first trimester screen has been available in Macedonia for several years, but only recently have been determined effective means of early chromosomal abnormality screening. In cases with chromosomal abnormalities we found a significant correlation between free beta human chorionic gonadotrophin hormone and nuchal translucency.
Conclusion: The screening of chromosomal abnormalities in pregnancy and the assessing risk of Down syndrome, Edward syndrome and Patay are of utmost importance for all pregnant women and the society as well.With this screening we are going to prevent their occurrence and we will reduce the psychological and physical suffering of parents and society, especially in today's modern society, where the technology is most advanced in the industry, and prevention is really possible!

Keywords: chromosomal anomalies, first-trimester, screening, pregnancy associated plasma protein-A

Introduction

Chromosomal anomalies are a leading cause of perinatal mortality and developmental abnormality. Consequently, the principal goal of prenatal testing is to screen for chromosomal anomalies and to provide genetic counseling for parents [1]. Trisomies 21, 18 and 13 and sex chromosome aberrations are the most frequently occurring chromosomal abnormalities.

First-trimester risk assessment of common chromosomal aneuploidy is based on a combination of maternal age, maternal serum free beta human chorionic gonadotrophin hormone, pregnancy associated plasma protein-A, and fetal nuchal translucency thickness [2].

The association between advancing maternal age and increased risk of trisomy 21 is well known, and pregnant women older than 35 years at delivery are routinely offered invasive prenatal diagnostic testing. The most commonly used test for genetic diagnosis is amniocentesis, but the rate of spontaneous fetal loss related to amniocentesis averages about one in every 200 procedures. Because of this risk, serum analytic testing has become an important, noninvasive first step in detecting patients at risk for congenital abnormalities [3].

First trimester screening is performed between first 10 and 14 weeks of the gestation. The markers used for the risk calculation are 2 serum markers: pregnancy associated plasma protein-Aand free beta human chorionic gonadotrophin hormone.

In 1974, pregnancy associated plasma protein-A was the one out of four proteins identified in the plasma of pregnant women [4]. Pregnancy associated plasma protein-A is produced in great amounts during pregnancy by the syncytiotrophoblast [5] and can be detected in placental tissue, decidua, maternal serum, amniotic and caulomic fluids [6, 7].

Maternal serum levels of pregnancy associated plasma protein-A, in the first trimester of pregnancy, are decreased in pregnancies with fetal trisomies 21, 18 or 13, dysgenictriploid,

monosomy X and those with impaired placentation resulting in pre-eclampsia and delivery of small for gestational age neonate.

In normal pregnancy, serum pregnancy associated plasma protein-A concentration is affected by gestational age and maternal characteristics, including: weight, racial origin, cigarette smoking, diabetes mellitus and method of conception [8].

Increased levels of free beta human chorionic gonadotrophin hormone are associated with an increased risk of Down syndrome.

The third marker is the fetal nuchal translucency which is performed by ultrasound. The nuchal translucency measurement needs to be performed by experienced sonographers and should be obtained between the first 10 and 13 weeks and 6 days of the gestation. The majority of fetuses with Down syndrome have an increase nuchal translucency measurement when compared to normal fetuses of the same gestational age [3]. Nuchal translucency is the sonographic appearance of a collection of fluid under the skin behind the fetal neck during the first trimester of pregnancy. An increased nuchal translucency is not only a marker for chromosomal anomalies, but also a nonspecific indicator of abnormal development, common to several pathologic pathways, including an increased risk of miscarriage or fetal death, from 1.6% in those with nuchal translucency between the 95th and 99th percentiles to approximately 20% for values above the 99th percentile, and a 15-fold increased likelihood of lethal or serious malformation [9].

The crown – rump length was obtained by measuring the fetal length from the tip of the cephalic pole to the tip of the caudal pole in the midsagittal plane [1].

Edward syndrome (trisomy [18] is the second most common form of chromosomal aneuploidy. The first trimester screening of trisomy 18 is based on the ultra-sonographic finding of nuchal translucency and decreases in maternal serum pregnancy associated plasma protein-A and free beta human chorionic gonadotrophin hormone. This test can detect 86% to 89% of cases with a 0.5% to 1.0% false-positive rate [1].

In Macedonia there is none published data on the association between serum biochemical and ultra-sonographic markers and adverse pregnancy outcomes. Consequently, the purpose of this study was to examine screening for chromosomal abnormalities in first trimester of pregnancy using ultrasound and maternal serum markers.

Methods

This prospective interventional study was performed between April 2015 and March 2017 in the Clinical Hospital "Dr. Trifun Panovski" in Bitola, Macedonia. A total number of 526 pregnant women were screened during the first trimester.

Maternal weight was measured using a digital weight scale eighth a precision of 0.1 kg. The serum was separated and pregnancy associated plasma protein-A and free beta human chorionic gonadotrophin hormone were measured using solid phase, enzyme labeled chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Inc., Llanberis, UK).

Transabdominal and transvaginal ultrasound examination was performed by certified maternal fetal medicine specialists. The ultrasound scan included a full structural survey, and nuchal translucency was measured according to established guidelines. Ultrasound examinations were performed with high resolution equipment (Voluson E Expert 2008, General Electric, Austria or Siemens G50 Ultrasound, Siemens Medical Solutions USA, Inc.)

Risks for chromosomal abnormalities were calculated using the software Prisca - mathematical model which gives individual risks for trisomy 21, 18 and 13. This mathematical model takes into consideration the maternal age, the serum levels of various biochemical markers and the fetus ultrasound measurements. In addition, a number of factors play an important role in the calculation of the risk as they will affect the values of the maternal serum biochemical analyzes. This includes: gestational age, weight, race, smoking, diabetic status of the individual, the number of fetuses present, and whether in vitro fertilization treatment was used for conceiving.

A calculated risk ≥1:250 were defined as high-risk for Down syndrome and ≥1:300 was defined as high-risk for Edward syndrome.

Results

Over a 2 - year period of time, screening was carried out in 526 pregnancies. Median maternal age was 29, 3 years old (range: 13, 4 to 43 years old), and 64 (12, 2%) of women who were 35 years old or older at the time of the assessment.

Table 1: Demographic characteristics of studied population (526 women, 535 fetuses)

Variable	Median (range) (%)	Total subjects
Maternal age (years)	29.3 (13.4-43)	462
≥ 35 years		64
Maternal weight (kg)	66,9(40 -208)	422
Not reported	19.7%	104
Mode of conception		526
spontaneous	96.7%	509
In vitro fertilization	1.5%	8
Not reported	1.7%	9
Smoking status		526
Smoker	9.5%	50
Non – smoker	88.8%	467
Not reported	1.7%	9
Racial origin		526

Caucasian	100%	526
Diabetes mellitus		526
Mother with diabetes mellitus	0.7%	4
Mother without diabetes mellitus	97.3%	512
Not reported	1.9%	10
Number of fetuses		526
singleton	98.3%	517
twins	1.7%	9
Gestational age at screening (days)	86.6 (70-97)	526
Crown – rump length (mm)	60.7(6.6 – 83.3)	526
Pregnancy associated plasma protein-A (mIU/ml)	3.9 (0.26- 19.4)	526
Free beta human chorionic gonadotrophin hormone (ng/ml)	44.6 (6.2- 170)	526
Nuchal translucency (mm)	0.89(0.14-4.73)	526

The median gestational age at screening was 86, 6 days or 12+2 gestational weeks (range: 70 to 97 days or 10 to 13+6 gestational weeks). The median crown – rump length was 60, 77 mm (range: 6, 6 to 83,3mm). The median maternal weight was 66, 94 kg (range: 40 to 108 kg).

Our study shows that smoker mother are 50, non-smokers are 467 and 9 were not reported. About the mode of conception, 502 was spontaneous, assisted with in vitro fertilization were eight, and nine were not reported.

Among the 526 pregnant women, four mothers were with diabetes mellitus, non-diabetes were 51, 2, and ten were not reported.

About the number of fetuses present, singleton pregnancies were 517 and nine were twins.

Table 2: Demographic characteristics of 48 cases with chromosomal abnormalities and 478 cases with a low risk of trisomy 21, 13, 18.

Variable	Median (range)(%) in unaffected pregnancies	Median (range)(%) in cases with chromosomal abnormalities
Maternal age (years)	29.08 (13.4-43)	32,2 (15,1 – 42,9)
Maternal weight (kg)	67.07 (41 - 108	65,6 (40 -90)
Not reported		
Mode of conception		
spontaneous	96.3%	97,9%
In vitro fertilization	1.7%	0
Not reported	1.7%	2,1%
Smoking status		
Smoker	9%	14,6%
Non – smoker	89.3%	83,3%
Not reported	1.7%	2,1%
Racial origin		
Caucasian	100%	100%
Diabetes mellitus		
Mother with diabetes mellitus	0.9%	0
Mother without diabetes mellitus	99.1%	97,9%
Not reported	0.9%	2,1%
Number of fetuses		
singleton	98.3%	97,9%
twins	1.7%	2,1%
Gestational age at screening (days)	86.6 (69-97)	86,4 (76 -95)
Crown – rump length (mm)	60.8 (6.6 – 83.3)	59,7 (39,9 – 77,9)
Pregnancy associated plasma protein-A (mIU/ml)	4 (0.37- 19.4)	2,41 (0,26 – 9,37)
Free beta human chorionic gonadotrophin hormone (ng/ml)	40.6 (6.2- 157)	95 (11,2 – 558)
Nuchal translucency (mm)	0.86 (0.14-2.02)	1,2 (0,26 – 4,73)

Among the 526 pregnant women overall, 48 (9, 1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. Out of the 526 women, 478 (90, 9 %) were cases with a low risk of trisomy 21, 13, 18, where chromosomal abnormality was not found.

In this interventional study we identified 48 cases of chromosomal abnormality: thirty eight of trisomy 21, nine of trisomy13/18. We identified 37 fetuses with biochemical T21 risk: six are with risk > 1:50; eight with risk 1: 50 –1:100; eight with risk 1: 100 –1:150; nine with risk 1: 150 –1:200 and 6 with risk 1:200 –1:250. Also, we identified 16 fetuses with combined trisomy 21 risk: eleven of them are with risk > 1:50; one with risk 1: 50 –1:100; three with risk 1: 100 –1:150 and one with risk 1: 150 –1:200.

Among the 526 pregnant women, nine (1, 7%) women had high risk for trisomy 13 or trisomy 18 using a cutoff value of 1:300. Three of them are with risk > 1:50; one with risk 1: 50 –1:100; two with risk 1: 100 –1:150; one with risk 1: 150 –1:200 and two with risk 1:200 –1:250.

Seven fetuses had biochemical trisomy 21 risk and combined

trisomy 21 risk. Thirty fetuses had just biochemical trisomy 21 risk. Nine fetuses had just combined trisomy 21 risk. Six are with combined trisomy 21 risk and trisomy 13/18 risk. One was with biochemical trisomy 21 risk, combined trisomy 21 risk and trisomy 13/18 risk. Two are just with trisomy 13/18 risk.

Table 3: Chromosomal abnormality frequencies

Chromosomal abnormality	n
Normal	478
Trisomy 21 and trisomy 13/18	48
Biochemical trisomy 21 rick	39
Biochemical trisomy 21 rick > 1:50	6
Biochemical trisomy 21 rick 1: 50 –1:100	8
Biochemical trisomy 21 rick 1: 100 –1:150;	8
Biochemical trisomy 21 rick 1: 150 –1:200	9
Biochemical trisomy 21 risk 1:200 –1:250	6
Combined trisomy 21 risk	16
Combined trisomy 21 risk > 1:50	11
Combined trisomy 21 risk1:50 –1:100	1
Combined trisomy 21 risk1:100 –1:150	3
Combined trisomy 21 risk1:150 –1:200	1
Trisomy 13 or trisomy 18	9
Trisomy 13 or trisomy 18 risk > 1:50	3
Trisomy 13 or trisomy 18 risk 1: 50 –1:100	1
Trisomy 13 or trisomy 18 risk 1: 100 –1:150	2
Trisomy 13 or trisomy 18 risk 1: 150 –1:200	1
Trisomy 13 or trisomy 18 risk 1:200 –1:250	2
Biochemical trisomy 21 risk and combined trisomy 21 risk	7
Just biochemical trisomy 21 risk	30
Just combined trisomy 21 risk	9
Combined trisomy 21 risk and trisomy 13/18 risk	6
Biochemical trisomy 21 risk, combined trisomy 21 risk and trisomy 13/18 risk	1
Just with trisomy 13/18 risk.	2

In group of fetuses with chromosomal abnormalities we found this correlations: a significant correlation between mother age and mother weight (p= 0,023; p<0, 05) and between mother age and free beta human chorionic gonadotrophin hormone (p= 0, 22); a significant correlation was established between gestational age and pregnancy associated plasma protein-A (p= 0,000; p<0, 01) and between gestational age and crown – rump length (p= 0,000). Also we found a significant correlation between mother weight and crown – rump length (p= 0,047; p<0, 05); we found a significant correlation between pregnancy associated plasma protein-A, and crown – rump length (p=0,000; p<0, 01) and we found a significant correlation between free beta human chorionic gonadotrophin hormone and nuchal translucency (p= 0,036; p<0, 05).

Discussion

The first trimester screen has been available in Macedonia for several years, but only recently have been determined effective means of early chromosomal abnormality screening. This screening is the most accurate, non-invasive screening method available. In this prospective study of first-trimester screening for chromosomal abnormalities by a combination of maternal serum biochemical markers and ultrasound markers in among the 526 pregnant women overall, 48 (9, 1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. Out of the 526 women, 478 (90, 9 %) were cases where chromosomal abnormality was not found.

In cases with chromosomal abnormalities we found a significant correlation between mother's age and mother's weight and between mothers' age and free beta human chorionic gonadotrophin hormone. A significant correlation was found between gestational age and pregnancy associated plasma protein-A, and between gestational age and crown – rump length. Also we found a significant correlation between mother weight and crown – rump length; we found a significant correlation between pregnancy associated plasma protein-A, and crown – rump length, and between free beta human chorionic gonadotrophin hormone and nuchal translucency.

The results of this study demonstrate that an enlarged nuchal translucency (above the 95th percentile) and advanced maternal age are associated with adverse perinatal outcomes in a mixed population (low- and high-risk), which is consistent with the findings reported in other studies [10, 11].

Measurement of serum pregnancy associated plasma protein-A may be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome. Effective use of serum pregnancy associated plasma protein-A in risk assessment and screening necessitates that variables from maternal characteristics and medical history which affect this measurement in normal pregnancy are taken into account. We found lower values of pregnancy associated plasma protein-A in cases with chromosomal abnormalities. About the values of free beta human chorionic gonadotrophin hormone we detect almost double higher values of free beta human chorionic gonadotrophin hormone in cases with high risk for chromosomal abnormalities compared between those with normal outcome. A positive correlation between high free beta

human chorionic gonadotrophin hormone and increased risk for chromosomal abnormalities is reported in other studies as well [12, 13].

In this study, we have outlined our first 2 - year experience in screening pregnancies. The limitation of this study is a small sample size in comparison with larger studies. Additionally, because this study was conducted in only one center, the result cannot fully represent the screening performance in Macedonian population.

In summary, the purpose of this study was to provide information on screening performance of the first trimester combined test, in a medical center of Bitola. To our knowledge, this study is the first population study analyzing the result of the first trimester combined test performed in Macedonia.

Conclusion

The screening of chromosomal abnormalities in pregnancy and the assessing risk of Down syndrome, Edward syndrome and Patay are of utmost importance for all pregnant women and the society as well.With this screening we are going to prevent their occurrence and we will reduce the psychological and physical suffering of parents and society, especially in today's modern society, where the technology is most advanced in the industry, and prevention is really possible!

Competing Interests

Authors have declared that no competing interests exist.

Authors Contributions

Author Biljana Ilkovska designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors Bisera Kotevska Trifunova, Georgi Trifunov, Marina Trajkovska and Branimir Kanazirev managed the analyses of the study. Author Sandra Hristovska managed the literature searches. All authors read and approved the final manuscript.

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