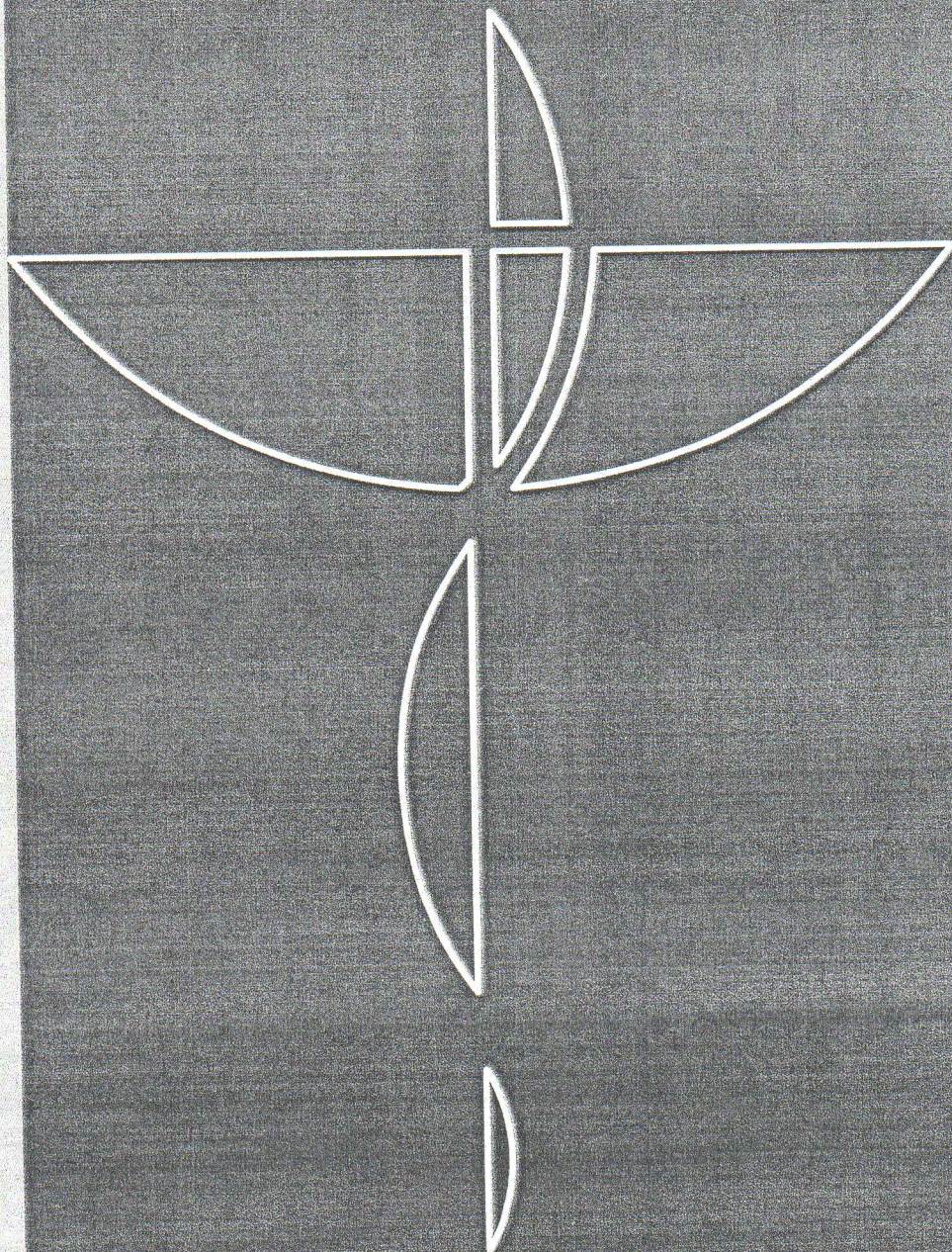


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# Physioacta





## LEPTIN LEVEL, BODY MASS INDEX AND LIPID PARAMETERS IN CHILDREN OF REPUBLIC OF MACEDONIA

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### Abstract

**Objective.** Leptin, an important signal in the regulation of adipose-tissue mass and body weight, operates by inhibiting food intake and stimulating energy expenditure. Overweight and obese youth are at increased risk for premature cardiovascular disease. It's well known that the development of atherosclerosis begins in childhood and is accelerated in the presence of obesity. Leptin plays an important role in the pathogenesis of obesity.

**Results.** Serum leptin levels were higher in overweight boys and girls compared to normal-weight boys and girls respectively (  $p < 0.001$ ;  $p < 0.001$  ). Serum leptin positively correlated with BMI in all examined groups. Triglycerides were statistically significant higher in overweight boys compared to normal-weight boys (  $p < 0.08$  ). Overweight girls had significantly higher LDL cholesterol (  $p < 0.006$  ) and significantly lower HDL cholesterol (  $p, 0.004$  ) than normal-weight girls.

**Conclusion:** Overweight boys and girls had higher leptin levels. The BMI showed a high correlation with leptin levels in all examined groups which suggests that BMI is the main indicator for the variations of leptin level. The concentration of LDL and HDL cholesterol differed only in the group of girls and triglycerides in the group of boys.

**Keywords.** Leptin, BMI, lipid parameters, children

## ВРЕДНОСТИ НА ЛЕПТИН, ИНДЕКС НА ТЕЛЕСНА МАСА И ПАРАМЕТРИ НА ЛИПИДЕН СТАТУС КАЈ ДЕЦА ОД РЕПУБЛИКА МАКЕДОНИЈА

### Апстракт

Цел. Лептинот, значаен сигнал во регулирањето на количината на масното ткиво и на телесната тежина, делува преку инхибиција на земањето на храна и преку стимулација на потрошувачката на енергијата.

Младите лица со прекумерна тежина и оние кои се гојазни се изложени на зголемен ризик за предвремени кардиоваскуларни болести. Добро е познато дека атеросклерозата започнува во детството и се забрзува во присуство на дебелината. Лептинот игра важна улога во патогенезата на дебелината.

Резултати. Нивото на лептинот беше повисок во серумот на момчињата и девојчињата со во поголема тежина во споредба со момчињата и девојчињата со нормална тежина ( $p > 0,001$ ;  $p < 0,001$  соодветно). Нивото на лептинот позитивно

корелира со БМИ во сите испитани групи. Триацилглицеролите беа статистички значајно повисоки кај момчињата со поголема тежина во однос на момчињата со нормална тежина ( $p < 0.08$ ). Кај девојчињата со поголема тежина утврдивме статистички значително повисоко ниво на LDL- холестерол ( $p < 0.006$ ) и статистички значително пониско ниво на ХДЛ –холестерол ( $p < 0.004$ ), во однос на девојчињата со нормална тежина.

Заклучок: Момчињата и девојчињата со прекумерна тежина имаат повисоко ниво на лептин. БМИ покажа статистички значителна висока корелација со нивото на лептинот во сите испитани групи, што значи дека БМИ е главен показател за варијациите на нивото на лептинот. Од липидните параметри, концентрацијата на LDL и на HDL холестеролот се разликува само во групата на девојки со прекумерна тежина во однос на оние со нормална тежина, а во групата момчиња разлика беше утврдена само во концентрацијата на триацилглицерглицеолите

**Клучни зборови .** Лептин, БМИ, липидни параметри, деца

### **Introduction:**

Leptin, discovered in 1994, is a 167- amino acid protein produced by the leptin gene (LEP), whose name is derived from the Greek word "leptos", which means "thin." Leptin, an important signal in the regulation of adipose-tissue mass and body weight, operates by inhibiting food intake and stimulating energy expenditure. Leptin is produced by the white adipose tissue, the most frequent form of adipose tissue in mammals. It is also produced in several other places including placenta, bone marrow, stomach, muscle, and perhaps brain, thus increasing the number of potential regulatory roles for this hormone. (1) The amount of body fat is the main determinant of the leptin circulating levels. (2) Leptin is transported across the blood-brain barrier. In the brain, leptin stimulates or inhibits release of several neurotransmitters such as neuropeptide Y, melanin-concentrating hormone, orexins alpha-melanocyte-stimulating hormone etc. (3) Defects in leptin production cause severe obesity.

(2) Obesity in child and adolescent population is a growing problem worldwide. Also, overweight and obese youth are at increased risk for premature cardiovascular disease. It is well known that the development of atherosclerosis begins in childhood and is accelerated in the presence of obesity. (4)

The aim of this study is to examine the concentration of serum leptin, BMI, and lipid parameters in normal- and overweight children of the Republic of Macedonia.

### **Materials and Methods**

This research was part of the cross-border project that was co-funded by the European Union and by the national funds of the participating countries under the IPA cross-border program "Greece – The Former Yugoslav Republic of Macedonia 2007-2013 "Using new technologies to promote children health in the cross-border region".

The study included 172 children (age  $10.94 \pm 1.56$  years) - 86 boys (age  $10.92 \pm 1.47$ ) and 86 girls (age  $10.77 \pm 1.53$ ) - with body mass index (BMI) ranging from 20 to  $41 \text{ kg/m}^2$ . A written informed consent was obtained from the parents of each child included in the study. Exclusion criteria were history of renal, hepatic, endocrine, and respiratory (with the exception of asthma) disease.



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First, the parents filled out a questionnaire about the physical activity of their child. The children had light indoor clothes and were barefooted during the measurement of their height and weight. Their standing height was measured with stadiometer to the nearest 0.1 cm. Weight was measured using a digital weight scale with a precision of 0.1 kg. BMI was calculated by dividing the weight by height squared ( $\text{kg}/\text{m}^2$ ). The authors used World Health Organization (WHO) growth reference data for children 5-19 years of age, which included weight, height, and BMI for these children. According to the BMI, the subjects were divided into four groups: normal weight girls normal weight girls boys with  $\text{BMI} < 25 \text{ kg}/\text{m}^2$ , and overweight boys and overweight girls with  $\text{BMI} > 25 \text{ kg}/\text{m}^2$ . (5) The blood samples were taken after overnight fast (12 hours). Lipid parameters, such as total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerols were measured in fresh sera by enzymatic methods, using biochemical analyzer Cobas Integra 400. Leptin was determined by ELISA kit (Mercodia Leptin ELISA, Sweden). The data are presented as mean  $\pm$  standard deviation (SD) and  $p \leq 0.05$  is considered statistically significant. The results were done with the SPSS version 16; statistical significance was test with paired Student's t-test, and Pearson correlation coefficient was used for correlation of BMI and leptin levels.

### Results

The concentration of leptin in all normal-weight children ranged from 2.07 ng/ml to 40.45 ng/ml (mean  $8.04 \pm 9.57$ ). However, statistical analysis showed that normal-weight girls had statistically higher leptin levels than normal-weight boys ( $p < 0.001$ ). Therefore, all 172 participants were divided in 4 groups: normal-weight (NW) girls, normal-weight (NW) boys, overweight (OW) girls and overweight (OW) boys ( $n=43$  each group). The anthropometric characteristics of each group are shown in Table 1.

**Table 1** Anthropometric characteristic of participants according to their sex and weight

Variables	NW-girls n=43	OW-girls n=43	P	NW- boys n=43	OW-boys n=43	P
Age ( years)	10,58 $\pm$ 0,98	10,95 $\pm$ 1,94	ns	10,51 $\pm$ 1,30	11,33 $\pm$ 1,54	ns
Body mass (kg)	43,28 $\pm$ 5,17	73,24 $\pm$ 17,20	0.001	42,97 $\pm$ 5,28	70,21 $\pm$ 15,73	0.001
Height ( m)	1.47 $\pm$ 1.20	1.49 $\pm$ 1.26	ns	1.51 $\pm$ 1.39	1.52 $\pm$ 1.48	ns
BMI( $\text{kg}/\text{m}^2$ )	21,84 $\pm$ 1,41	29,70 $\pm$ 3,79	0.005	21,98 $\pm$ 1,47	27,88 $\pm$ 4,35	0.005
Waist circumference (cm)	60,48 $\pm$ 9,91	76,3 $\pm$ 7,21	0.001	59,51 $\pm$ 8,91	73,61 $\pm$ 6,32	0.005
Hip circumference (cm)	76,78 $\pm$ 8,91	83,42 $\pm$ 9,36	0.001	72,79 $\pm$ 8,37	80,15 $\pm$ 8,96	.002

NW -normal weight; OW- overweight ;  $p > 0.05$ -statistical significant



Table shows that BMI, waist and hip circumference are statistically significantly higher in overweight girls ( $p < 0.005$ ;  $p < 0.001$ ; and  $p < 0.001$ ) and overweight boys ( $p < 0.005$ ;  $p < 0.005$ ;  $p < 0.00$ ), compared to normal-weight girls and boys, respectively.

Serum leptin levels significantly correlate with BMI in normal-weight and overweight girls (Fig. 1 A and B) and in normal-weight and overweight boys (Fig. 2 A and B), respectively.

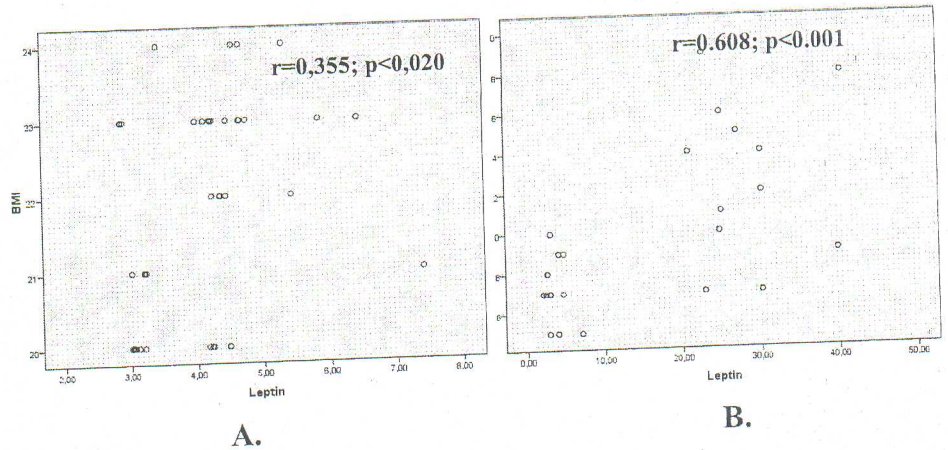


Figure 1 Correlation between leptin level and BMI in normal weight girls ( $r=0.355$ ;  $p<0.020$ ) (A) and overweight girls ( $r=0.608$ ;  $p<0.001$ ) (B)

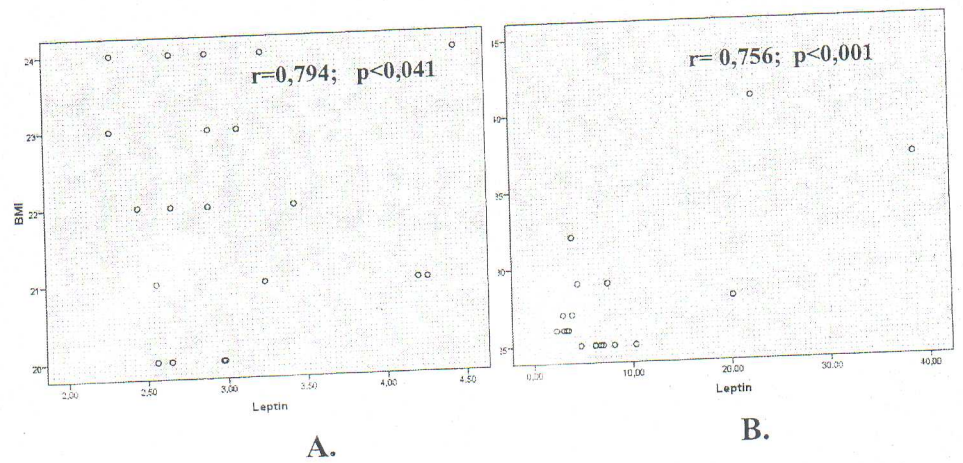


Figure 2 Correlation between leptin level and BMI in normal weight boys ( $r=0.794$ ;  $p<0.041$ ) (A) and overweight boys ( $r=0.756$ ;  $p<0.001$ ) (B)



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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-A and 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, dysgenitriplod,



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  $\geq 1:250$  were defined as high-risk for Down syndrome and  $\geq 1:300$  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
$\geq 35$ years		64
Maternal weight (kg)	66,9(40 -208)	422
Not reported	19.7%	104
Mode of conception		526
spontaneous	96.7%	509
<i>In vitro</i> 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%
<i>In vitro</i> 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 trisomy 13/18. 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 risk	39
Biochemical trisomy 21 risk > 1:50	6
Biochemical trisomy 21 risk 1: 50 –1:100	8
Biochemical trisomy 21 risk 1: 100 –1:150;	8
Biochemical trisomy 21 risk 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 risk 1:50 –1:100	1
Combined trisomy 21 risk 1:100 –1:150	3
Combined trisomy 21 risk 1:150 –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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