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The significance of maternal blood pregnancy-associated plasma protein A (PAPP-A) and free beta-subunit of human chorionic gonadotropin (β-hCG) levels for the risk assessment of fetal trisomy 18 during the first prenatal testing between 11 and 13^{+6} weeks of pregnancy

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ABSTRACT

Objective: The aim of the study was to evaluate the significance of the maternal blood level of pregnancy-associated plasma protein A (PAPP-A) and free beta-subunit of human chorionic gonadotropin (β-hCG), to estimate the risk of fetal trisomy 18 and their correlation with the assessment of nuchal translucency (NT) during the first prenatal testing.

Material and methods: Examinations of 93 pregnant women between 11 and 13^{+6} weeks of pregnancy were conducted, which included determination of β-hCG and PAPP-A concentrations in the maternal serum and ultrasound assessment of fetal nuchal translucency. Concentrations of biochemical parameters were expressed as multiples of median (MoM) for the appropriate gestational age. The risk assessment of trisomy 18 was analyzed using Astraia software. Pregnant women with a high (≥ 1:300) risk of trisomy 18 were offered a genetic amniocentesis with an examination of fetal karyotype. Twenty cases were healthy and 23 with trisomy 18.

Results: PAPP-A and β-hCG MoM values < 0.3 were found in 61% cases of fetal trisomy 18. In 26% of cases, PAPP-A and β-hCG MoM values < 0.2 were NT-independent risk factors for trisomy 18. There were no significant differences between groups with normal fetal karyotype (40%) and trisomy 18 (35%) in PAPP-A and β-hCG MoM 0.2–0.5 range.

Conclusions: Maternal free β-hCG MoM was found to change parallelly to fetal NT widening in case of trisomy 18 diagnosis. Maternal β-hCG and PAPP-A MoM results presented less then 0.2 might be used independently of NT widening in fetus for trisomy 18 risk evaluation. Above 0.2 for PAPP-A and β-hCG MoMs, fetal NT measurement was an requirment.

Key words: trisomy 18, pregnancy-associated plasma protein A (PAPP-A), free beta-subunit of human chorionic gonadotropin (β-hCG), nuchal translucency

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INTRODUCTION

Prenatal screening based on non-invasive diagnostics allows to determine the risk of chromosome aberrations in the fetus and limit the use of invasive procedures [1–5]. Since the 1990s, the diagnostic scheme for pregnant women between 11 and 13^{+6} weeks of pregnancy has been in force, consisting of the evaluation of free beta-subunit of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum, as well as an ultrasonographic evaluation of fetal anatomy with measurement of nuchal translucency (NT), which may contribute to the detection of 94% cases of trisomy 18

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[5, 6–12]. Results of PAPP-A and β-hCG concentrations are converted into multiples of the median (MoM) by dividing the concentration of the examined parameter by the median for a given week of pregnancy. In a normal pregnancy, the concentration of PAPP-A increases exponentially, while the concentration of β-hCG, after the initial increase, decreases between 10 and 14 weeks of pregnancy [5]. A PAPP-A MoM decrease below 0.500 on average, along with β-hCG MoM decrease below 0.300 in maternal serum is considered to characterize trisomy 18 [7–20].

The discussion on the advisability of concurrent ultrasound and biochemical tests and their role in the diagnosis of chromosome aberrations has been an ongoing topic for over 20 years [5]. In this study, we analyzed the values of MoM for PAPP-A and β-hCG to be essential for calculating the risk of Edwards' syndrome (trisomy 18) occurrence and how fetal neck translucency ultrasound assessment influences the effectiveness of the syndrome diagnosis in the first trimester of pregnancy.

The aim of the study was to evaluate the significance of the maternal blood level of biochemical parameters: PAPP-A and free β-hCG, to estimate the risk of fetal trisomy 18 and their correlation with the assessment of NT during the first prenatal testing.

MATERIAL AND METHODS

The study included 93 pregnant women from the Prenatal Diagnostic Center of Gynecology and Obstetrics Hospital of Poznan University of Medical Sciences, who were assessed for the risk of chromosome aberrations occurrence between 11 and 13^{+6} weeks of pregnancy. The study included ultrasonographic evaluation of fetal anatomy, including NT and fetal heart function, as well as the measurement of PAPP-A and β-hCG concentrations in maternal serum.

Then in 43 pregnant women with increased risk of trisomy 18 (≥ 1:300) occurrence, genetic amniocentesis was performed, in accordance with the recommendations of the Ultrasonography Division of the Polish Society of Gynecologists and Obstetricians. The control group consisted of 50 pregnant women with a low (< 1:1000) risk of trisomy 18 occurrence. In the group with increased risk of trisomy 18 occurrence, the mean age was 34.17 years (from 19 to 43) and body mass index (BMI) — 23.79 (SD ± 3.35), while in the control group — 33 years (from 17 to 41) and BMI — 23.86 (SD ± 4.5) respectively, and did not differ statistically.

The ultrasound examination was performed using the Voluson E8. GE instrument (USA) and included evaluation of fetal anatomy and heart function, along with measurement of fetal NT as a marker of risk of chromosome aberrations (trisomy 21, 18, 13) — according to Fetal Medicine Foundation standards and based on recommendations of the Polish Society of Gynecologists and Obstetricians, Ultrasonography Division [1, 2, 5, 6, 21].

The concentrations of PAPP-A and β-hCG in maternal serum were evaluated using the immunofluorescence (time-delayed fluorescence) method, by means of the Delfia Xpress device (PerkinElmer Life and Analytical Sciences, Waltham, USA) [5, 12].

The calculation of the risk of chromosome aberrations, including biochemical and ultrasound markers, was performed using the ©2000–2016 Astraia software (Astraia Software Gmbh, Munich, Germany) [1, 5, 6, 21].

Amniocentesis was performed after taking about 20 mL of amniotic fluid. Amniocytes were cultured *in vitro* after obtaining cytogenetic slides, following by karyotype analysis utilizing the metaphase G-banding method.

The analysis of PAPP-A and β-hCG MoMs was carried out in both studied groups by dividing the concentration of the examined parameter by the median for a given week of pregnancy, with following ranges determined for them: 0.001–0.200; 0.201–0.300; 0.301–0.500; > 0.501.

The NT values of the fetuses in the group with trisomy 18, that were classified into the predefined ranges of: 1.0–2.0 mm, 2.1–3.0 mm, 3.1–5.0 mm and > 5.1 mm.

The statistical analysis was performed using the PQStat software. The variable distribution normality was analyzed by Shapiro-Wilk tests. Groups were compared with Mann-Whitney's U test, and analyzed with Pearson correlation coefficient and receiver operating characteristic (ROC) curves.

RESULTS

In 93 cases examined, an increased (≥ 1:300) risk of trisomy 18 occurrence was observed in 43 cases with genetic amniocentesis performed. Edwards syndrome was reported in 23 cases, while in 20 pregnant women the fetal karyotype was normal. The number of pregnant women over 35 years old was evaluated in the study groups: 60.87% of subjects with trisomy 18 diagnosed in newborns (n = 23), 75% of subjects at increased risk but normal fetal karyotype in newborns (n = 20) and 58% of controls (n = 50). In the analyzed groups, the pregnant woman's age had no significant impact on the results of the tests, and this parameter was not determined as an evaluation criterion.

In the group of patients with diagnosed fetal trisomy 18 (n = 23), lower values of β-hCG MoM and PAPPA-A MoM were observed, in comparison to patients with healthy fetuses (n = 70) (Fig. 1, 2).

Analysis of ROC curves for the assessed classifiers: PAPP-A MoM and β-hCG MoM allowed to distinguish pregnant women with fetal trisomy 18 in the examined material (Fig. 3, 4). PAPP-A MoM of 0.154 was indicated as a cut-off value with 98.6% sensitivity and 43.5% specificity estab-

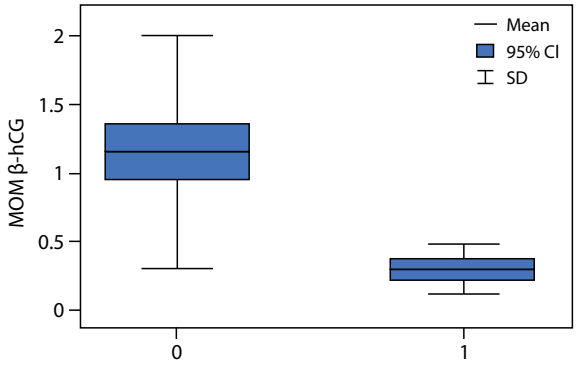

Figure 1. Comparison of free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) in the patient group with fetal trisomy 18 (1) and in the group with with a normal karyotype (0)

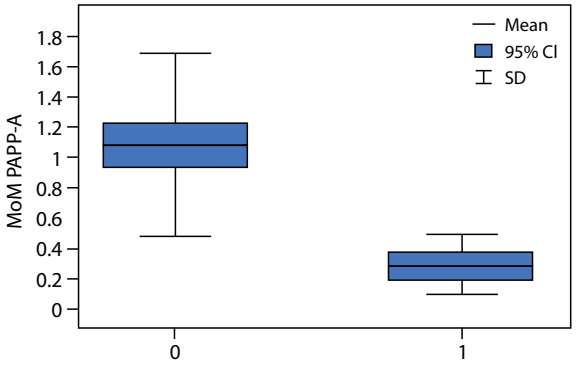

Figure 2. Comparison of associated plasma protein A (PAPP-A) multiples of median (MoM) in the patient group with fetal trisomy 18 (1) and in the group with a normal karyotype (0)

Table 1. Analysis of receiver operating characteristic curve parameters for pregnancy-associated plasma protein A (PAPP-A) multiples of median (MoM) and free beta-subunit of human chorionic gonadotropin (β-hCG) MoM — comparison of pregnant women with fetal trisomy 18 (n = 23) and pregnant women with a normal fetal karyotype (n = 70)

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	p
PAPP-A MoM	0.154	98.6	43.5	0.849	< 0.001
β-hCG MoM	0.369	95.7	69.6	0.892	< 0.001

AUC — area under the curve; p — statistical significance

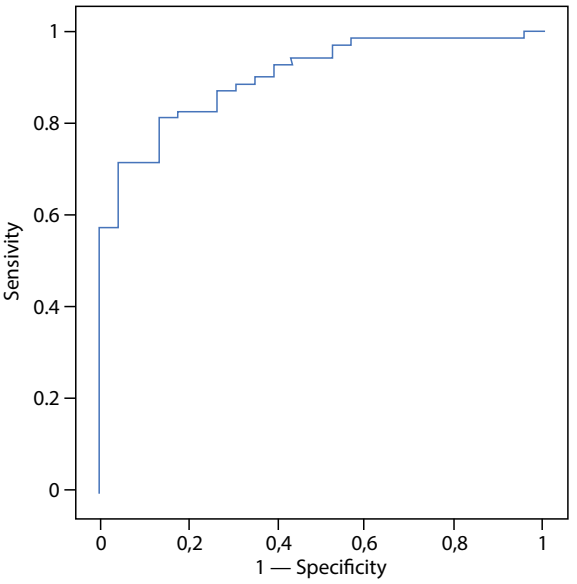

Figure 3. Receiver operating characteristic curve — sensitivity and specificity for associated plasma protein A (PAPP-A) multiples of median

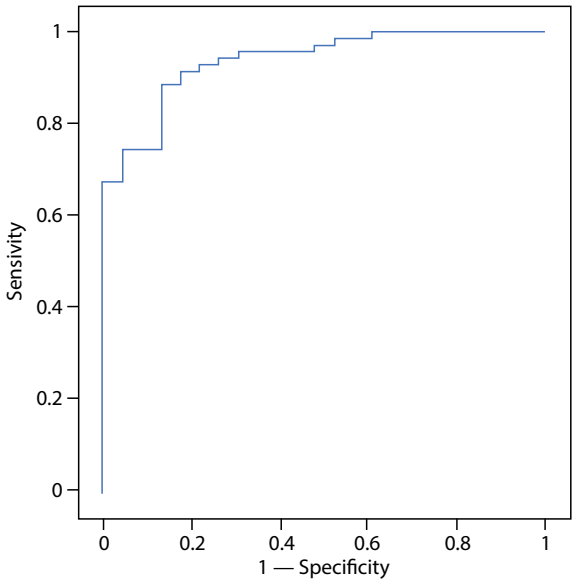

Figure 4. Receiver operating characteristic curve — sensitivity and specificity for free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median

lished. As the PAPP-A MoM decreases from the cut-off point, the sensitivity increases and the probability of diagnosing trisomy 18 increases. β-hCG MoM of 0.369 was assumed as a cut-off point with 95.7% sensitivity and 69.6% specificity established. A decrease of β-hCG MoM from the cut-off point increases the probability of diagnosing trisomy 18 (Tab. 1, Fig. 3, 4).

In the group of pregnant women with fetal trisomy 18, PAPP-A MoM concentration values were 0.277 ± 0.215 and were lower than the group with normal fetal karyotype — 0.720 ± 0.677 ($p < 0.05$).

The PAPP-A MoM analysis showed that the highest percentage of patients with low (< 1:1000) trisomy 18 risk was in the > 0.501 MoM range and was 96%. The high-

est percentage of patients with fetal trisomy 18 (52%) was in the < 0.2 PAPP-A MoM range, and the lowest (13%) in > 0.501 MoM, however, 35% of patients with fetal trisomy 18 and 40% of pregnant women with normal fetal karyotype were in the 0.2–0.5 PAPP-A MoM range, which indicate light differences between these groups (Fig. 5).

The mean value of the β-hCG MoM in the group of pregnant women with fetal trisomy 18 (0.298 ± 0.180) was lower than in the group with normal fetal karyotype (1.04 ± 1.232) ($p < 0.05$). Detailed analysis of β-hCG MoM showed that — similarly to PAPP-A MoM values — 13% of pregnant women with fetal trisomy 18 were presented with > 0.500 β-hCG MoM and 39.13% of them were presented with < 0.200 MoM of β-hCG. In the control group (described by < 1:1000 risk of fetal trisomy 18) 94% of pregnant women were found > 0.500 MoM of β-hCG and 6% of them were found within 0.301–0.500 MoM of β-hCG. However, 47.8% of pregnant women with fetal trisomy 18 and 35% of pregnant women with normal fetal karyotype were presented with 0.200–0.500 MoM range of β-hCG, adequately to similar results obtained for PAPP-A MoMs (Fig. 6).

The mean value of NT in the group of pregnant women with fetal trisomy 18 was 4.66 ± 2.514 mm (median 4.4) and was higher compared to the group with normal fetal karyotype, 1.836 ± 0.308 mm ($p < 0.05$) (median 1.8).

The correlation between NT and biochemical parameters PAPP-A MoM and β-hCG MoM was analyzed in the study groups.

The positive relationship between NT and β-hCG MoM ($R = 0.40$, $p = 0.012$) was observed only in pregnant women with fetal trisomy 18 (Fig. 7).

Detailed analysis of NT values within selected PAPP-A MoM ranges showed that in pregnant women with fetal trisomy 18 presented with < 0.200 PAPP-A MoM 26% of subjects were found NT > 3.1 mm and 26% of them were found NT < 3.1 mm, which may suggest higher diagnostic

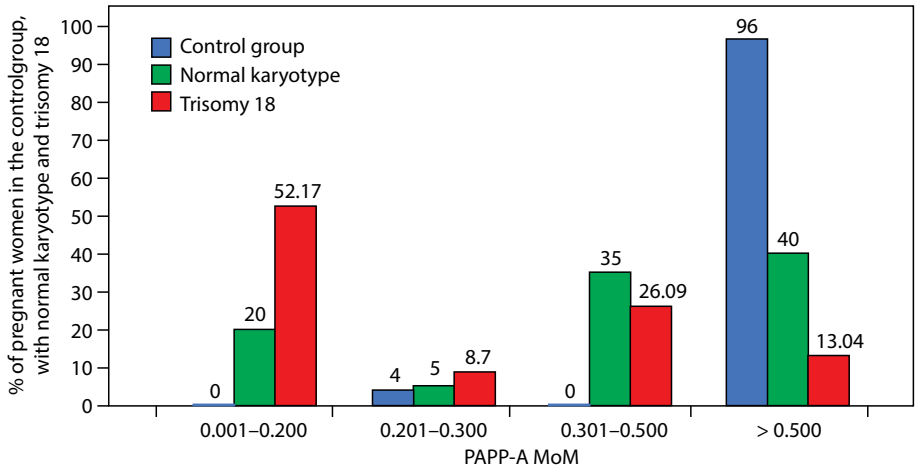

Figure 5. Percentage of pregnant women in the control group, a group with increased risk of trisomy18 but normal fetal karyotype and confirmed trisomy 18 within the analyzed associated plasma protein A (PAPP-A) multiples of median (MoM) ranges

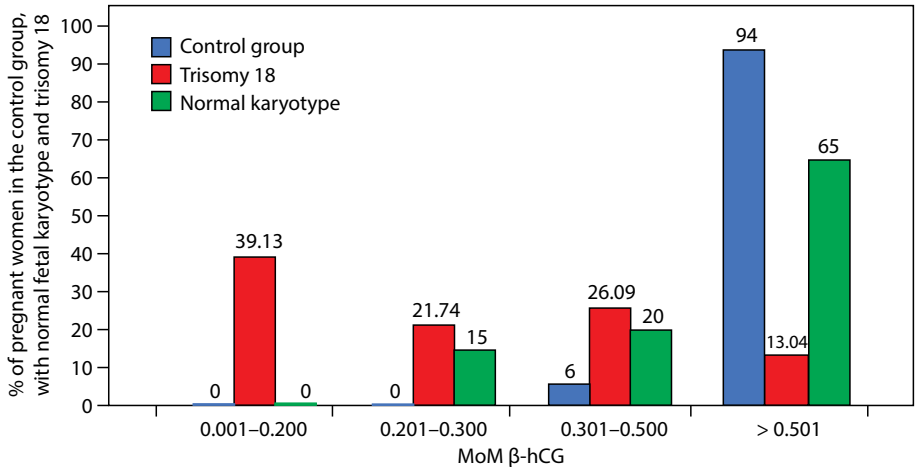

Figure 6. Percentage of pregnant women in the control group, group with increased risk of trisomy 18 but normal fetal karyotype and confirmed trisomy 18 in the analyzed free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) ranges

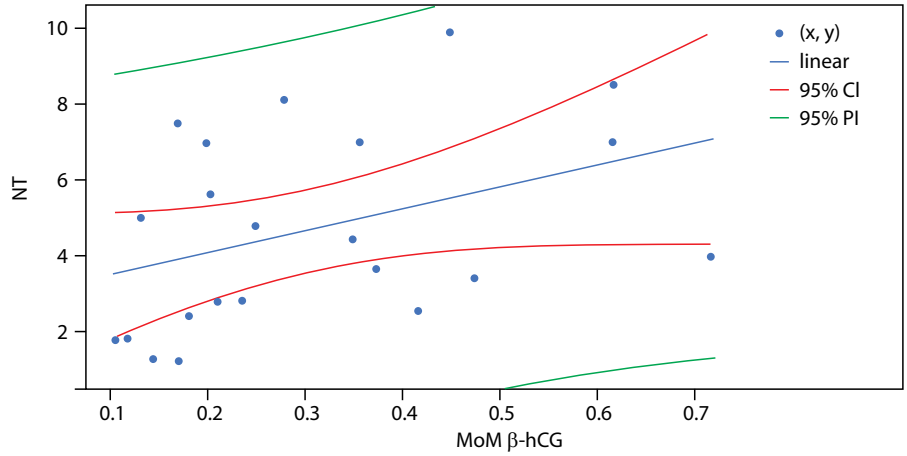

Figure 7. Correlation analysis between free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) and fetal nuchal translucency (NT) in a group with prenatally diagnosed fetal trisomy 18

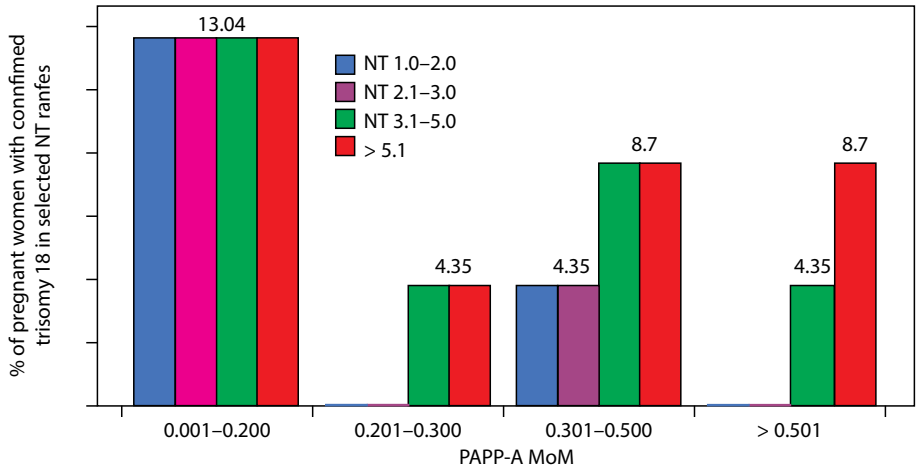

Figure 8. Nuchal translucency (NT) analysis in selected associated plasma protein A (PAPP-A) multiples of median (MoM) ranges in the group of pregnant women with diagnosed fetal trisomy 18

value of biochemical parameters in the analyzed range. However, in the PAPP-A MoM 0.201-0.300 range, all fetuses had a widening of nuchal translucency (> 3.1 mm). It was also observed that in the >0.501 PAPP-A MoM range (normal values), 13% of fetuses with trisomy 18 had a NT widening, which was the only test parameter that determined an increased risk of trisomy 18 (Fig. 8).

In the group of pregnant women with fetal trisomy 18 and with β-hCG MoM values < 0.200, nuchal transparency < 3.1 mm was observed in 26.1% of cases, and only 13% of fetuses had widening of the NT, which may indicate higher diagnostic value of biochemical parameters within this range. In the 0.201–0.500 MoM range, the majority (34.8%) of fetuses had widened NT. In contrast, in the > 0.500 β-hCG MoM range (normal values) in 13% of fetuses, a widening of the nuchal translucency was observed, which (similarly to PAPP-A) was the only test parameter determined the increased risk of trisomy 18 (Fig. 9). This indicates that the β-hCG MoM and PAPP-A MoM values are independent of the nuchal translucency values, but instead complement each other. It is also worth noting that the lower the MoM values of biochemical parameters PAPP-A and β-hCG, the lower the impact of neck translucency on the risk assessment of fetal trisomy 18. The analysis of median ranges of PAPP-A and free β-hCG demonstrates that none of the analyzed parameters is sensitive enough to give an unambiguous answer about the possibility of trisomy 18 occurrence.

DISCUSSION

Non-invasive, first-trimester prenatal screening encompassing a combination of maternal serum biochemistry assays (β-hCG and PAPP-A) and ultrasound-assessed NT enables accurate identification of approximately 90% of chromosomal abnormalities with 5% false positive results [5, 6, 8, 9, 20]. The components of the first trimester testing do not always correlate with each other in accordance with

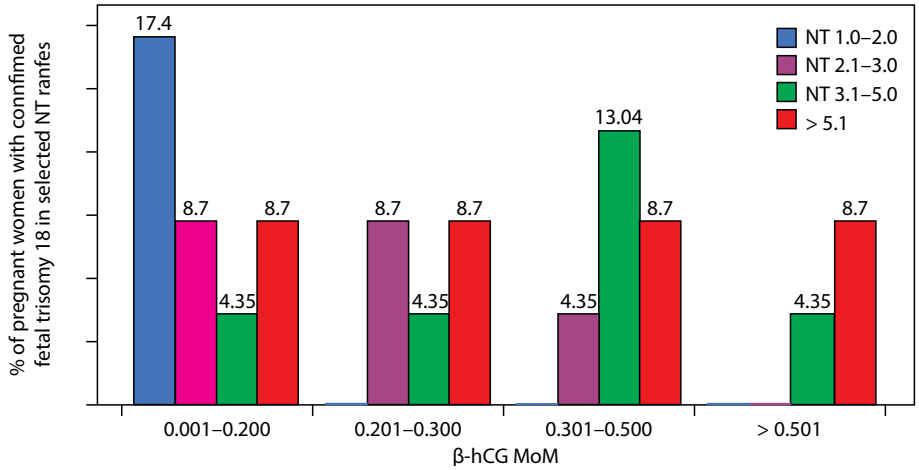

Figure 9. Nuchal translucency (NT) analysis in selected free beta-subunit of human chorionic gonadotropin (β-hCG) multiples of median (MoM) ranges in the group of pregnant women with diagnosed fetal trisomy 18

accepted standards, but ultimately the test result indicates an increased risk of fetal malformation. The analysis showed that the mean β-hCG MoM and PAPP-A MoM in the group of pregnant women with fetal trisomy 18 did not differ significantly from the reports of other authors [7–20].

The distribution of trisomy 18 in particular β-hCG MoM and PAPP-A MoM ranges was analyzed. The study confirmed that the majority of cases of prenatally diagnosed trisomy 18 met the criteria for each of the examined parameters, 61% for < 0.300 PAPP-A and β-hCG MoM. The analysis also showed the diagnostic value of β-hCG and PAPP-A parameters in the < 0.200 MoM range being independent of the fetal nuchal translucency widening in the group of patients with fetal trisomy 18. However, it is worth noting that between the group of pregnant women with normal fetal karyotype results (40%) and one with trisomy 18 (35%) there were no differences in PAPP-A MoM and β-hCG MoM values within 0.200–0.500 MoM range. This not only indicates does not only indicate a small low diagnostic value of biochemical parameters in aforementioned PAPP-A and β-hCG MoM range, but emphasizes the role of the quality of testing. The method of storing blood intended for testing blood storage conditions prior to analysis and the quality of the devices the type of laboratory equipment for determining these parameters using to establish small differences of PAPP-A and β-hCG levels in these groups, may be necessary for the calculation of the final risk of fetal trisomy 18. This particularly applies to the concentration of β-hCG, which incorrect storage results in an increase in its levels, diminishing the diagnostic value of this parameter in the final risk assessment. The analysis of biochemical parameters in this range clearly showed that an additional ultrasound assessment of nuchal translucency is necessary for the correct assessment of the risk of trisomy 18 occurrence in the first prenatal examination.

The correlation of the examined biochemical parameters and measurement of nuchal translucency showed that the characteristic decrease in PAPP-A MoM and β-hCG MoM < 0.3 and widening of NT occurred only in 22% of pregnant women, similarly to reports of other authors [7–9, 15]. In 22% of cases of prenatally diagnosed trisomy 18, there was no widening of nuchal translucency, therefore only the biochemical parameters confirmed the effectiveness of the first trimester testing [20]. On the other hand, in 13% of cases the results of biochemical tests were normal, and only the widening of nuchal translucency decided about an increased risk in the test calculation. All cases with prenatally confirmed fetal trisomy 18 demonstrated an increased risk in the test. This confirms that the principle of the screening test is to select a group with an increased risk for Edwards syndrome occurrence [5].

CONCLUSIONS

Of the biochemical parameters analyzed in the study, maternal β-hCG MoM was found to change parallelly to fetal NT widening in case of trisomy 18 diagnosis. Taking into account the ranges of PAPP-A and β-hCG MoM values used in obstetric practice for the aneuploidy risk assessment, maternal β-hCG and PAPP-A MoM results presented less then 0.200 might be used independently of NT widening in fetus for trisomy 18 risk evaluation.

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