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The clinical usefulness of biochemical (free β-hCG, PAPP-A) and ultrasound (nuchal translucency) parameters in prenatal screening of trisomy 21 in the first trimester of pregnancy

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ABSTRACT

Objectives: The aim of the study was to analyze the correlation of multiples of the normal median of PAPP-A, free β-hCG levels and nuchal translucency values in prenatal, first trimester screening of trisomy 21 in pregnant women.

Material and methods: 251 pregnant women underwent antenatal screening at 11–13^{+6} weeks of pregnancy which was composed of the measurement of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP-A) levels in the maternal serum and an ultrasound assessment of nuchal translucency (NT). The pregnant women with a high risk of trisomy 21 (≥ 1:300) were given amniocentesis to verify fetal defects. There were 217 cases of normal fetal karyotype and 34 cases of trisomy 21. PAPP-A, β-hCGMoM and NT values were analyzed for the predefined ranges.

Results: 85% cases of trisomy 21 had elevated free β-hCGMoM (> 1.5) and only 53% of these had a PAPP-AMoM result below 0.5 ($p < 0.05$). Analysis of NT in selected ranges of β-hCG (> 1.5) and PAPP-AMoM (< 0.05), which are typical for Down Syndrome values, showed that not all fetuses with Down Syndrome presented with an increased NT. Respectively 44.15% and 26.5% of fetuses presented with increased NT. Characteristic for trisomy 21, a correlation with all 1st trimester screening tests' parameters occurred in only 23.5% of cases. In 53% of cases the results were atypical.

Conclusions: The PAPP-A and β-hCG values in the selected MoM ranges did not shown a correlation to the NT measurement, therefore they are independent factors in the diagnosis of trisomy 21. Simultaneous biochemical and ultrasound testing is an indispensable condition for prenatal diagnosis of trisomy 21 in the 1st trimester of pregnancy.

Key words: PAPP-A; free β-hCG; nuchal translucency (NT); prenatal screening; trisomy 21

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INTRODUCTION

Prenatal screening that include non-invasive diagnostic tests enable assessment of the risk of fetal chromosomal aberrations and may reduce the use of invasive procedures associated with the 0.5–1.0% risk of miscarriage [1–4]. Since the 1990s, measuring maternal serum free beta human chorionic gonadotropin (β-hCG) and pregnancy associated plasma protein (PAPP-A) levels has been included in first-trimester prenatal screening carried out at 11–13^{+6} weeks of gestation alongside a fetal ultrasound assessment that includes nuchal translucency (NT) measurement. This, according to different authors, enables the detection of approximately 80–90% of all trisomy 21 cases [1, 2, 5–11]. The measured concentrations of either PAPP-A or free β-hCG are converted into the multiples of the median (MoM) appropriate to the gestational age of each pregnancy. The MoM value is obtained by dividing

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an individual's marker concentration by the median level of that marker for the entire population at the same gestational age in that laboratory. In a healthy pregnancy, the maternal serum PAPP-A level increases exponentially, whereas the free β-hCG level drops, after an initial increase, in the period between 10 and 14 gestational weeks [12]. According to FMF and Polish Society of Gynecologists and Obstetricians recommendations, a PAPP-A MoM below 0.5, free β-hCG MoM above 1.5, and a nuchal translucency increase in weeks 11–13^{+6} of gestation are typical of fetal trisomy 21 [7–11, 13–15].

The debate on the utility of combined ultrasound and biochemistry testing and the role of such screening in detecting chromosomal aberrations has been ongoing for over 20 years. Therefore, a detailed analysis was conceived to assess the association between the individual parameters and to determine whether the magnitude of PAPP-A level reduction and free β-hCG level elevation are of clinical significance in Down Syndrome risk calculation.

Aim

The aim of the study was to analyze the correlation in multiples of the normal median of biochemical parameters in maternal serum — PAPP-A and free β-hCG — as well as nuchal translucency values in the 11–13^{+6} weeks of gestation in patients with cytogenetically confirmed trisomy 21.

MATERIAL AND METHODS

The study included a group of 251 pregnant women aged 18 to 46 years (mean age of 35.9 years), with a mean BMI of 23.44, who were patients at the Ultrasonography and Prenatal Imaging Clinic of the Gynaecology and Obstetrics Hospital of Poznan University of Medical Sciences. All women with an increased risk of trisomy 21 (≥ 1:300) in the 1st trimester screening test (double test), had genetic amniocentesis to assess the fetal karyotype, in accordance with recommendations of the Ultrasonography Division of the Polish Society of Gynecologists and Obstetricians.

Assessment of the occurrence of chromosomal aberrations was carried out using the routine antenatal scan at 11–13^{+6} gestational weeks, which consisted of ultrasound fetal assessment with a maternal serum assay of PAPP-A and free β-hCG.

Ultrasound examination included assessment of the risk markers of the most common chromosomal aberrations (trisomy 21, 18, 13) — crown-rump length (CRL) of 45–85 mm, nuchal translucency (NT), fetal heart rate, and blood flow in the ductus venosus with fetal anatomy and chorion [1, 2, 16].

Maternal serum concentrations of free β-hCG and PAPP-A were determined using an immunofluorometric assay on the Delfia Xpress analyzer (Perkin-Elmer Life and Analytical Sciences, Waltham, USA). The reaction surface was coated with specific antibodies directed against the respective PAPP-A and β-hCG antigen determinant. Then, antibodies to other antigenic determinants of the parameters were studied, labeled with fluorochrome (Europium), and added to be able to read the concentrations of the determined parameters. The resulting complex was read at 612 nm.

The risk of trisomy 21 was calculated based on the measured biochemical markers and ultrasound parameters using ©2000–2016 Astraia software (Astraia Software Gmbh, Occamstr. 20, 80802 Munich, Germany) [1, 13, 16].

Participants with an elevated risk of trisomy 21 (< 1:300) were offered amniocentesis in line with the recommendation of the Ultrasonography Division of the Polish Society of Gynecologists and Obstetricians. It consisted of abdominal collection of approximately 20 mL of amniotic fluid, then amniocytes — amniotic fluid cells were cultured in a special media. In the cytogenic preparations obtained after the culture, metaphases colored by the G-band method were analyzed to assess the karyotype.

Patients were sub-divided into two groups according to the occurrence of fetal defects: group I — pregnant women with a normal karyotype; and group II — pregnant women with trisomy 21 in fetuses.

In both groups a detailed analysis of the PAPP-A and free β-hCG MoM values was carried out to determine their correlation with the risk of trisomy 21. The following PAPP-A MoM ranges were determined: 0.001–0.500; 0.501–0.900; and above 0.901. Similarly, the following free β-hCG MoM ranges were determined: 0.001–1.000; 1.001–1.500; 1.501–2.000; and above 2.000.

The nuchal translucency (NT) values of the fetuses in the group with trisomy 21, that were classified into the predefined ranges of 1.0–2.0 mm, 2.1–3.0 mm, 3.1–5.0 mm, 5.1–8.0 mm, and above 8.1 mm, were analyzed for PAPP-A MoM of 0.001–0.500 and above 0.500 and free β-hCG MoM of 0.001–1.500 and above 1.500.

Statistical analysis was carried out using the PQStat bundle. Normality of distribution was verified using the Kolmogorov–Smirnov test, Lillefors test and Shapiro-Wilk test. The ROC analysis of the assessed classifiers (PAPP-A MoM and β-hCG MoM) enabled us to distinguish between participants with fetal trisomy 21 and those with normal fetal karyotype.

RESULTS

Of all patients who underwent cytogenetic evaluation, 217 were diagnosed with normal fetal karyotype and 34 cases were diagnosed with trisomy 21. In the group with normal karyotype results, the patients ≥ 35 years old accounted for 68.8% of the trisomy 21 cases and 31.2% were < 35 years. The age distribution was similar in the group with fetal trisomy 21, with 67.6% of the women aged ≥ 35 years and 32.3% aged < 35 years. In the study group overall, the age of the pregnant women had no significance on the results of the study and this parameter was discarded as an assessment criterion.

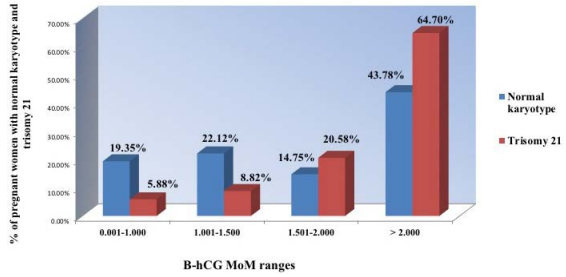

Figure 1. Cases of fetal euploidy and trisomy 21 in the analyzed free β-hCG MoM ranges

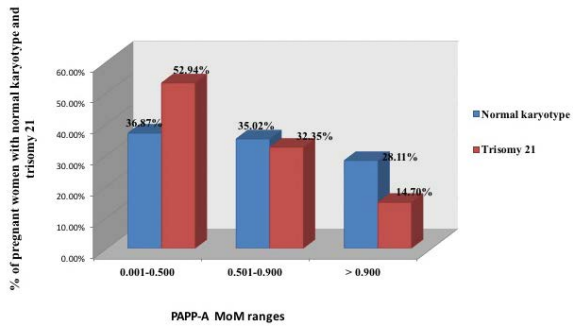

Figure 2. Cases of fetal euploidy and trisomy 21 in the analyzed PAPP-A MoM ranges

The mean β-hCG MoM = 2.894, with SD ± 1.665 in 34 cases of fetal trisomy 21, was higher than in the group with normal karyotype results β-hCG MoM = 1.979, with SD ± 1.569 ($p < 0.05$).

A detailed analysis of free β-hCG MoM demonstrated that the maternal serum free β-hCG MoM was above 1.5 in most of the fetal trisomy 21 cases (85.28%). At the same time, 58.53% of the participants with free β-hCG MoM above 1.5 had normal fetal karyotype, including 43.78% of the cases with free β-hCG MoM above 2.0 (64.70% for fetuses with trisomy 21).

Cases of confirmed fetal trisomy 21 constituted only a small part (14.7%) of the subgroup with free β-hCG MoM below 1.5. 41.50% were cases with normal fetal karyotype (Fig. 1).

The mean PAPP-A MoM in cases of confirmed fetal trisomy 21 was significantly lower than in cases of fetal euploidy (0.539 ± 0.281 vs. 0.691 ± 0.45, $p < .05$).

The PAPP-A MoM analysis demonstrated the largest proportion (52.94%) of trisomy 21 cases in a subgroup with PAPP-A MoM ranging between 0.001 and 0.500. At the same time, the euploidy cases comprised 36.87% of that subgroup.

A subgroup with PAPP-A MoM above 0.501 consisted of trisomy 21 cases in 47.05% of the subjects and euploidy cases in 63.13% of the subjects (Fig. 2).

The ROC analysis for the assessed classifiers (PAPP-A MoM and β-hCG MoM) enabled us to distinguish between participants with fetal trisomy 21 and those with normal fetal karyotype (Fig. 3 and 4).

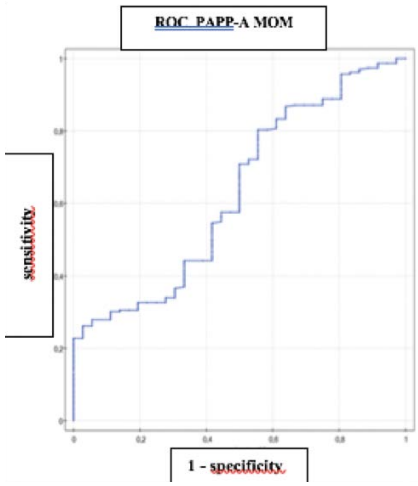

Figure 3. ROC curve - sensitivity and specificity of PAPP-A MoM to distinguish between cases of trisomy 21 and euploidy

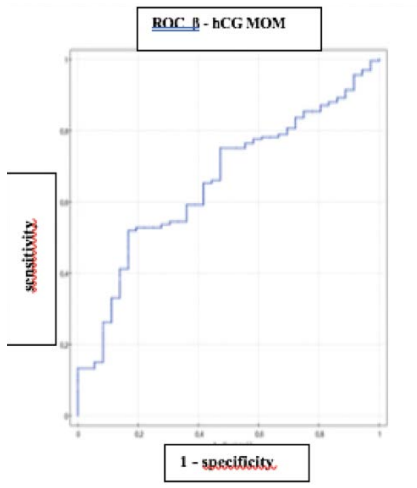

Figure 4. ROC curve - sensitivity and specificity of β-hCG MoM to distinguish between cases of trisomy 21 and euploidy

The cut-off value of PAPP-A MoM was 0.463. Its sensitivity and specificity were 70.8% and 50%, respectively. A decrease of PAPP-A MoM below the cut-off value increases the marker's sensitivity and the likelihood of trisomy 21. The cut-off value of free β-hCGMoM was 2.17. Its sensitivity and specificity were 75.1% and 52.8%, respectively. An increase in the free β-hCG MoM above the cut-off value increases the likelihood of trisomy 21 (Tab. 1).

In cases of confirmed fetal trisomy 21, nuchal translucency was significantly higher than in cases of euploidy (3.9 mm ± 1.893 vs. 2.2 mm ± 0.938).

The next stage was to analyze the fetal nuchal translucency in selected PAPP-A MoM ranges. It was demonstrated that among the cases of known fetal trisomy 21, only 26.5% of fetuses had NT over 3.1 mm if the maternal serum PAPP-A MoM fell within the range 0.001-0.500.

Table 1. ROC analysis — (cut-off point, sensitivity, specificity, area under the curve — AUC, statistical significance level — p) PAPP-A MoM and β-hCG MoM for classifiers differentiating between participants with fetal trisomy 21 and normal fetal karyotype

Variable	Cut-off point	Sensitivity [%]	Specificity [%]	AUC	p
PAPP-A MoM	0.463	70.8	50.0	0.63	0.013
β-hCGMoM	2.71	75.1	52.8	0.656	0.003

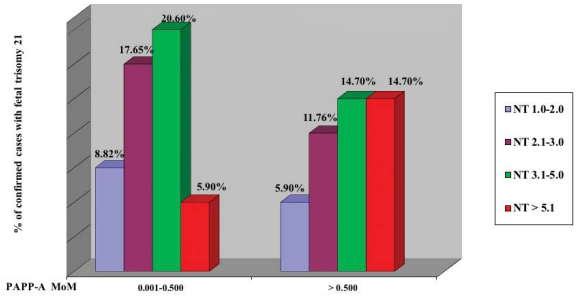

Figure 5. NT analysis for selected PAPP-A MoM ranges in participants with known fetal trisomy 21

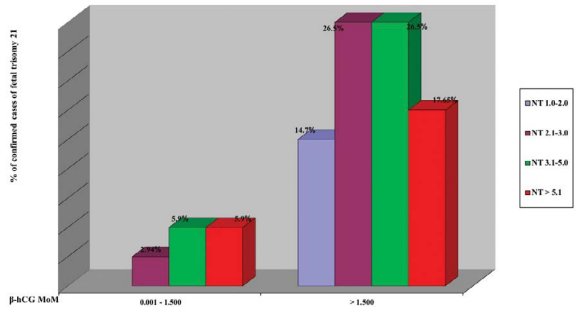

Figure 6. NT analysis for selected bHCG MoM ranges in participants with known fetal trisomy 21

Also, 26.5% of fetuses with known trisomy 21 and maternal serum PAPP-A MoM below 0.500 did not present with increased NT; instead, PAPP-A MoM was the only aneuploidy indicator.

Furthermore, 17.66% of fetuses with known trisomy 21 and maternal serum PAPP-A MoM above 0.500 did not present with increased NT as compared with 29.4% of fetuses with NT over 3.1 mm in the same subgroup. Among these cases, only the increased NT sub-set was indicative of Down Syndrome risk (Fig. 5).

Among the confirmed cases of fetal trisomy 21, in a subgroup of participants with free β-hCG MoM over 1.5, an NT increase over 3.1 mm was demonstrated in 44.15% of cases, as compared with 41.2% of cases with an NT below 3.0 mm. On the other hand, in a subgroup of participants with free β-hCGMoM below 1.5, an NT increase over 3.1 mm was demonstrated in 11.8% of cases, as compared with 2.94% of cases with an NT below 3.0 mm (Fig. 6).

The combined analysis of biochemical parameters and nuchal translucency in the group with fetal trisomy 21 showed that 23.5% of pregnant women had PAPP-A MoM < 0.5 and > 1.5 β-hCG MoM with an increased NT. Also, 23.5% of cases of this group presented with typical trisomy 21 biochemical parameters without an NT increase.

Among the confirmed trisomy 21 cases, there were 18 cases (53%) of atypical PAPP-A and free β-hCG MoM values, combined with the absence of simultaneous PAPP-A MoM reduction and free β-hCG MoM elevation, which typically indicates a high risk of Down Syndrome. A combination of normal PAPP-A MoM (of about 1.0) and elevated free β-hCGMoM (above 1.5) was shown in 38.2% (n = 13) of known trisomy 21 cases; and in addition, 61.5% of these fetuses had an increased nuchal translucency. A combination of normal free β-hCG MoM and reduced PAPP-A MoM (below 0.5) was shown in 8.8% of known trisomy 21 cases; and in addition, 66.7% of these fetuses had an increased nuchal translucency. The two remaining cases of trisomy 21 had reduced PAPP-A MoM combined with reduced free β-hCGMoM, and normal PAPP-A MoM combined with reduced free β-hCGMoM, respectively. Nuchal translucency was increased in both cases. (Tab. 2 and 3).

The presented results prove that the biochemical values and nuchal translucency, as parameters, are independent of each other in the diagnosis of trisomy 21.

DISCUSSION

Non-invasive, first-trimester prenatal screening encompassing a combination of maternal serum biochemistry assays (free β-hCG and PAPP-A) and ultrasound-assessed nuchal translucency (NT) enables accurate identification of approximately 90% of chromosomal abnormalities with 5% false positive results [7, 8, 13, 16]. The results of individual tests may not always correlate with each other as per the current standards; however, the ultimate output will indicate an increased risk of aneuploidy. We have observed this in our sample of known trisomy 21 cases. The preliminary data analysis showed no significant differences between the mean free β-hCG and PAPP-A MoM levels obtained in our sample of known trisomy 21 cases and those reported by other authors [8, 9, 12, 14, 17].

The main aim of our study was to determine the distribution of trisomy 21 cases in individual free β-hCG and PAPP-A MoM ranges. A detailed analysis indicated that most cases of trisomy

Table 2. Atypical PAPP-A MoM, β-hCG MoM and NT in pregnant women with diagnosed fetal trisomy 21

No.	PAPP-A MoM	free B-hCG MoM	NT	Karyotype	Double marker test risk estimate
1.	0.885	5.468	6	47, XY, + 21	1 : 2
2.	0.844	1.750	5.5	47, XY, + 21	1 : 12
3.	0.334	1.236	8	47, XY, + 21	1 : 2
4.	0.568	1.095	2.5	47, XX, + 21	1 : 108
5.	1.035	3.924	6.0	47, XX, + 21	1 : 2
6.	0.676	0.715	6	47, XX, + 21	1 : 3
7.	0.904	9.304	2.7	47, XX, + 21	1 :4
8.	0.782	1.895	4	47, XX, + 21	1 : 2
9.	0.947	2.714	8.6	47, XY, + 21	1 : 4
10.	0.915	1.858	2.5	47, XY, + 21	1 : 128
11.	0.973	0.788	4.8	47, XY, + 21	1 : 4
12.	0.837	2.053	4.2	47, XX, inv(9)(p12q13), + 21	1 : 4
13.	0.806	3.773	4	47, XY, + 21	1:4
14.	0.839	3.547	1.6	47, XX, + 21, 21 pss	1 : 29
15.	0.318	1.442	5	46, XX, der(14; 21)(q10; q10), + 21	1 : 2

Table 3. Atypical PAPP-A and free β-hCG MoM values in pregnant women with confirmed fetal trisomy 21

PAPP-A and free β-hCGMoM value analysis	No. of cases with trisomy 21	% of all trisomy 21 cases detected antenatally
normal PAPP-A MoM and free β-hCGMoM ≥ 1.5	13	38.2
PAPP-A MoM < 0.5 and normal free β-hCGMoM	3	8.8
decreased both PAPP-A MoM and free β-hCGMoM	1	2.9
normal PAPP-A MoM and decreased free β-hCGMoM	1	2.9

21 detected during antenatal screening, as seen in previous studies, had free β-hCGMoM over 1.5 (85.28%), but only 53% of these had PAPP-A MoM below 0.5. The correlation of all components of the characteristics in the screening test for the risk of trisomy 21 (< 0.5 PAPP-A MoM and > 1.5 β-hCG MoM) and increased NT, occurred in only 23.5% of the pregnant women. In all cases with prenatally diagnosed 21 fetal trisomy, the evidence of risk was increased in the screening test. Our findings prove the benefit of antenatal screening in helping to identify high-risk cases for Down Syndrome [18].

The analysis of free β-hCG and PAPP-A MoM values in cases with known fetal trisomy 21 identified 53% (n-18) atypical cases with biochemical test results and their configuration different from those typically seen in fetal trisomy 21. The most common atypical configuration was a normal PAPP-A MoM (of approximately 1.0) combined with an elevated free β-hCGMoM (above 1.5). Most fetuses in this subgroup presented with an increased NT. In such cases, the free β-hCGMoM and increased NT were the indicators of trisomy 21. Furthermore, there were 2.9% cases with an atypical configuration involving PAPP-A MoM below 0.5 combined with a normal free β-hCGMoM; and increased NT was demonstrated in most of these fetuses. In the two remaining cases, the biochemical parameter MoM values were not indicative of trisomy 21. However, their abnormal concentrations and an increased NT indicated they were high risk and led to the ultimate confirmation of Down Syndrome in the fetuses.

Additionally, we have analyzed in detail the nuchal translucency data in the selected free β-hCG and PAPP-A MoM ranges. Although the mean NT in a subgroup with known fetal trisomy 21 was 3.9 mm, which is consistent with previously reported findings [8, 9, 19], not all fetuses in this subgroup presented with an increased NT. In those latter cases, the elevated risk of trisomy 21 was primarily determined by abnormal maternal serum concentrations of free β-hCG and/or PAPP-A.

Our findings unequivocally indicate that none of the analyzed parameters on their own offers enough sensitivity to provide a conclusive finding of fetal trisomy 21. In our sample, there were cases of confirmed Down Syndrome with normal maternal serum concentrations of assessed biochemical parameters as well as those with normal NT. The analysis shows that maternal serum biochemical markers and ultrasound fetal screening are complementary and should not be considered separately in trisomy 21 diagnostics.

CONCLUSIONS

1. The analysis of maternal serum PAPP-A and free β-hCG levels along with ultrasound-based nuchal translucency measurement are independent of each other as parameters in trisomy 21 diagnostics.

2. Assessing biochemical and ultrasound parameters in combination is an indispensable condition of assessing the risk of trisomy 21 occurrence in antenatal screening during the first-trimester.

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