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ORIGINAL RESEARCH

The Levels of Pregnancy-Associated Plasma Protein (PAPP-A) and Chorionic Gonadotropin (β-hCG) in the Blood Serum of Women with Hypothyroidism in the 1st Trimester of Pregnancy

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Introduction: Hypothyroidism occurs in pregnant women at a rate of 0.3% to 3%. The deficiency of thyroid hormones during pregnancy can lead to an increased risk of pregnancy complications and poor health of the child, particularly affecting its psychomotor development due to the intensive growth of the nervous system during gestation. The study attempted to establish the median concentrations of pregnancy-associated plasma protein PAPP-A and the free subunit of human chorionic gonadotropin β-hCG in women with hypothyroidism in the 1st trimester of pregnancy.

Objective: The study attempted to establish the median concentrations of pregnancy-associated plasma protein PAPP-A and the free subunit of human chorionic gonadotropin β-hCG in women with hypothyroidism in the 1st trimester of pregnancy.

Patients and methods: The study included 210 pregnant women between 11 and 13.6 weeks of pregnancy; 105 women were diagnosed with hypothyroidism before or during pregnancy, and 105 women of a similar body weight and gestational age had normal thyroid function.The measurements of the pregnancy parameters studied were performed using the DELFIA® Xpress system.

Results: Differences in the multiples of the median of the PAPP-A and β-hCG levels between women with hypothyroidism and healthy women were observed.

Conclusion: Introducing correction for patients with hypothyroidism during non-invasive biochemical prenatal testing may allow obtaining more reliable results that would be the basis for referral to invasive procedures.

Keywords: pregnancy-associated plasma protein, human chorionic gonadotropin, pregnancy, hypothyroidism

Introduction

Thyroid hormones increase oxygen consumption, which translates into increased metabolism and has a fundamental impact on human growth and development.[1] Deficiency of thyroid hormones is particularly dangerous for the fetus during pregnancy and for the newborn because of the intensive development of the nervous system in that period.[2,3] It may also increase the risk of pregnancy complications and poor health of the child, particularly affecting its psychomotor development.[4,5] Hypothyroidism occurs in pregnant women at a rate of 0.3% to 3%.[6,7]

Thyroid physiology changes during pregnancy, similarly to most organs in the body of a pregnant woman. The production of thyroid hormones increases by 50% in the first weeks of pregnancy. This is caused by human chorionic gonadotropin (hCG).[4,5,8] hCG and thyroid stimulating hormone (TSH) belong to glycoprotein hormones; their α subunits are identical, and there are small differences in their β subunits, which allows hCG to affect thyroid cells in a largely similar manner to TSH. These properties are relatively weak, but because the concentration of hCG in the 1st trimester of

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pregnancy is very high, this action is very significant. Free β-hCG is formed as a result of partial degradation of the entire molecule under the influence of the blood serum enzyme – elastase. During pregnancy, free β-hCG appears in the maternal serum earlier than the A subunit, peaking at 9–12 weeks of gestation (later than all hCG). The level of hCG increases rapidly after fertilization, reaches its peak by the end of the 1st trimester, and subsequently decreases to reach a stable level in approximately 20th week of pregnancy.[4,8,9]

Another reason for which the thyroid increases its hormone production is the increased capacity of the main protein transporting thyroid hormones, thyroxine-binding globulin. In order to maintain homeostasis and the normal free hormone concentration, the production of hormones needs to be increased. What is more, thyroid hormones are inactivated by type 3 deiodinase, and a certain amount of maternal hormones (mainly thyroxine) passes into the fetus. Iodine is needed to increase the production of thyroid hormones.[4,10] In central Europe, pregnant women can easily develop iodine deficiency as its supply at the population level is only satisfactory. Moreover, iodine is eliminated from the body because of increased renal clearance and transfer to the fetoplacental unit.[11,12]

Non-invasive screening, with precise anatomical evaluation of the fetus including markers of aneuploidy, in conjunction with free β-hCG and PAPP-A biochemistry, has become an integral part of prenatal diagnostics in pregnant women. It allows calculating the individual genetic risk of aneuploidy.[13–15] However, additional factors, such as smoking, medications, body weight, in vitro fertilization, type 1 diabetes, may influence the levels of the parameters tested. As a result, a higher percentage of false results can be expected.

Therefore, it is important to identify the factors influencing the final result and to include them in the estimation of genetic risk.[16]

Aim of the Study

The aim of the study was to assess how hypothyroidism affects the plasma levels of PAPP-A and the free subunit of β-hCG in women with hypothyroidism in the 1st trimester of pregnancy, as well as the complete evaluation of genetic risk based on a non-invasive combined prenatal test.

Materials and Methods

The study group comprised pregnant patients of the Diagnostic and Medical Centre "Lipowa" Sp. z o. o. in Bydgoszcz, Poland, where a detailed patient interview was conducted by a physician specialized in clinical genetics. Subsequently, a prenatal ultrasound (US) examination was conducted and a blood sample collected. Blood analyses are detailed in Table 1.

The study was performed in accordance with the Declaration of Helsinki and accepted standards of ethics. The consent of the relevant bioethics committee was obtained: Bioethics Committee of Nicolaus Copernicus University, Toruń, Poland, N^{o} 551/2019.

Material

The tested material was approximately 5 mL of peripheral blood taken into a vacuum tube with gel serum separator. Subsequently, the levels of PAPP-A and the free subunit of β-hCG in the sample were determined. The DELFIA® Xpress immunoanalysis and biochemistry system, used for prenatal screening, was employed in the study.

Table 1 Study Group

Study Group	Number	Age (Years)	Body Weight (Kg)	Smoking	CRL (mm)	Gestational Age (Week)
STUDY GROUP	105	32 (23–41)	65 (44–119)	3	63.42 (45.2–83.2)	11–13
CONTROL GROUP	105	32 (23–41)	66 (48–119)	0	62.54 (47.5–78.3)	11–13

Notes: Study group – women diagnosed with hypothyroidism before or during pregnancy. Control group – women of a similar body weight and gestational age without hypothyroidism.

Methods

Determination of the biochemical markers, PAPP-A and the free subunit of β-hCG, was performed between 11 and 13.6 weeks of pregnancy, employing a fully automated random access analytical system, which measures delayed fluorescence for each parameter tested. The obtained concentrations were analysed by calculating multiples of the median (MoM) for selected pregnancy parameters. Genetic risk was calculated based on a prenatal US examination conducted by a certified physician, as well as biochemical tests run on an audited FMF system coupled with the Astraia software.

Statistical calculations and visualization of the results were done using the Statistica and Microsoft Excel software.

Results

Once the medians of the plasma concentrations of PAPP-A and the free subunit of β-hCG were calculated, the obtained pregnancy parameters were examined for atypical (outlying) observations. For this purpose, two box plots were generated: one for the study group and one for the control group Figure 1.

Each group contained one extreme value which was rejected from further analysis Figure 2.

In addition, a slightly greater number of atypical observations could be noted in the study group. At this stage, it was questionable whether the observed pattern confirmed the hypothesis of the effect of hypothyroidism on β-hCG.

In the study group, the mean value of the β-hCG MoM was higher, and the standard deviations in both groups were different Table 2.

In order to better analyse the results collected, Student's *t*-test was performed for the two means, and the statistical significance of the differences was tested. Two hypotheses were taken into consideration:

Null hypothesis: The mean values of β-hCG MoM are the same in the group of women with hypothyroidism diagnosed before or during pregnancy and in the group of women of a similar body weight and gestational age without hypothyroidism.

Alternative hypothesis: The mean values of β-hCG MoM are different in both groups Table 3.

Because $p > 0.05$, the following null hypothesis should be considered: The mean values of β-hCG MoM are equal. However, it cannot be assumed that the test performed is correct because the *p* value for variance is less than 0.05, and therefore the assumption for Student's *t*-test that the variance is homogeneous is not met. The above steps were repeated for the PAPP-A MoM in Figure 3.

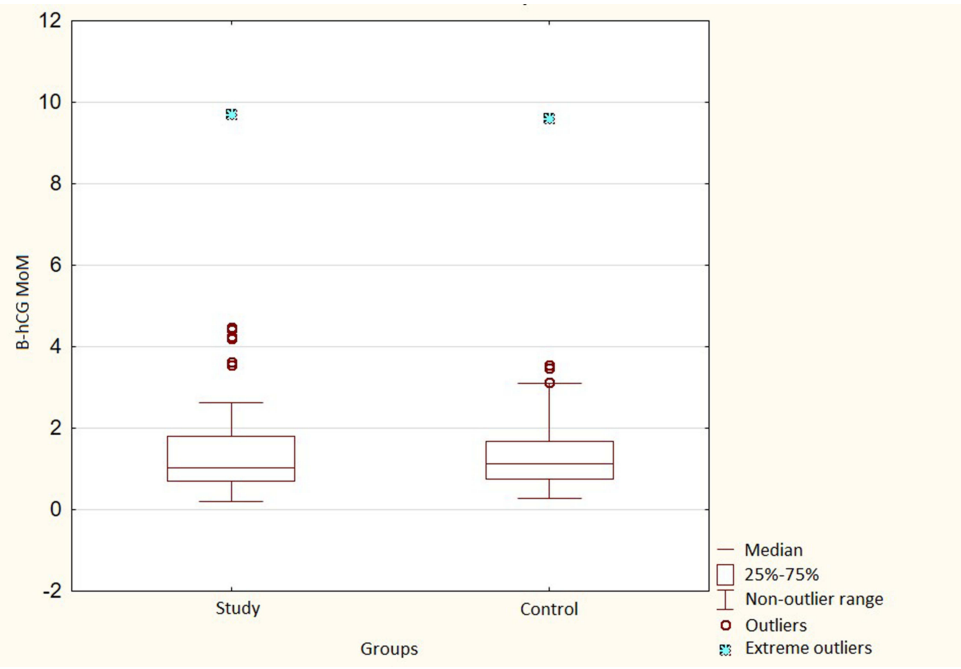

Figure 1 β-hCG MoM box plots in the study group and the control group.

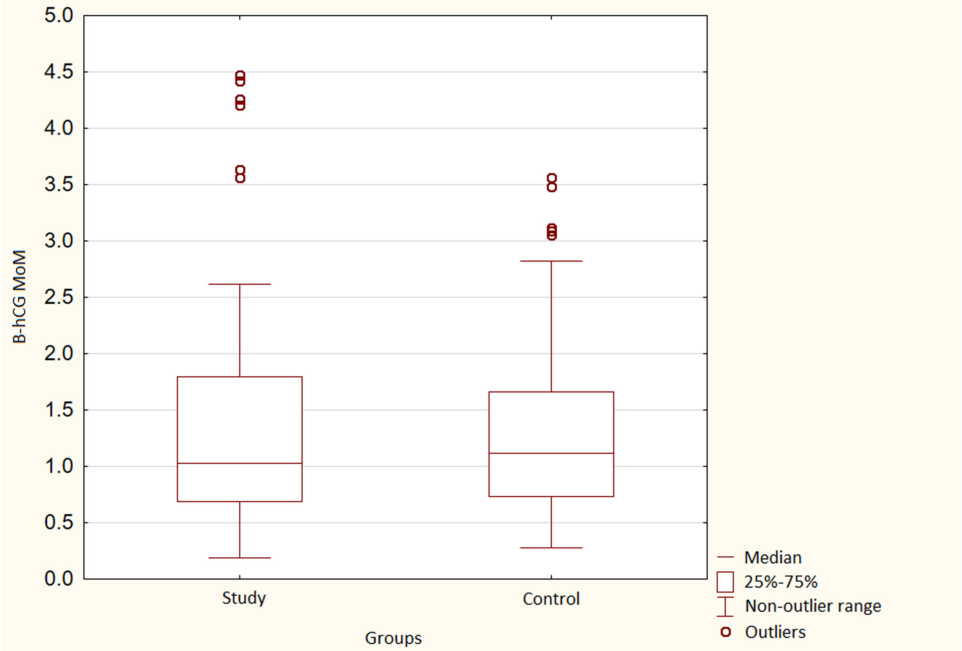

Figure 2 Box charts following rejection of outliers.

For this parameter, no outliers warranting rejection were found, but some more atypical observations were again made in the study group compared with the control group Table 4.

As with the β-hCG MoM parameter, the mean value was found to be higher in the study group, and Student's *t*-test for two means was run in order to determine the statistical significance of the difference between these means.

Two hypotheses were taken into consideration:

Null hypothesis: The mean values of PAPP-A MoM are the same in the group of women with hypothyroidism diagnosed before or during pregnancy and in the group of women of a similar body weight and gestational age without hypothyroidism.

Alternative hypothesis: The mean values of PAPP-A MoM are different in both groups Table 5.

In this case, the variance values in both groups were similar (homogeneous), which suggests that the assumptions of Student's *t*-test for the two means were met. Because the obtained *p* value was higher than 0.05 (0.452), the null hypothesis should be rejected, and the alternative hypothesis (ie the mean values are different in both groups) should be considered.

Table 2 Basic Statistics of β-hCG MoM Following Rejection of Outliers

Group	Group	Mean	Minimum	Maximum	Standard Deviation
Study group	104	1.372	0.19	4.473	0.957
Control	104	1.297	0.28	3.560	0.757

Notes: Study group – women diagnosed with hypothyroidism before or during pregnancy. Control group – women of a similar body weight and gestational age without hypothyroidism.

Table 3 Results of Student's t-test of the Two Mean β-hCG MoM Values

	Sample	t	df	*p*	*p* Variance
B-hCG MoM	104	0.629	206	0.530	0.018

Notes: t – Student's t-distribution. df – number of degrees of freedom. *p* – (*p*-value) test probability. *p* variance – (*p*-variance) – test probability for variance (Levene's test).

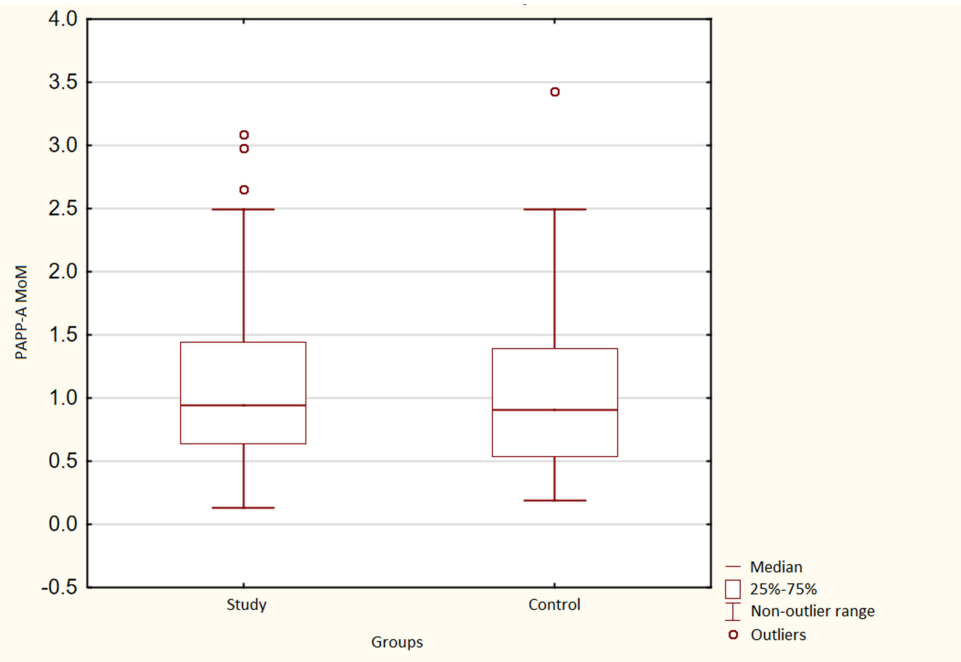

Figure 3 PAPP-A MoM box plots in the study group and the control group.

The basic statistics of the β-hCG MoM and the PAPP-A MoM parameters allowed us to conclude that patients with hypothyroidism have a greater tendency for increased median plasma concentrations of PAPP-A and the free subunit of β-hCG than women without hypothyroidism. Although abnormalities were found in the β-hCG mean values test in the study group, special attention should be paid to the increased number of atypical observations in patients of the study group.

Subsequently, additional factors were analysed which may have been relevant to the values of the parameters examined, ie patient age and body weight.

In both groups, women were divided into different age ranges [(23–25>), (25–30>), (30–35>), (35–41>) years] in which the mean values of the β-hCG MoM and the PAPP-A MoM were analysed Figure 4.

The study group (women diagnosed with hypothyroidism before or during pregnancy) had higher mean values of the β-hCG MoM at age ranges (23–25>) and (30–35>) years. For patients aged (25–30>), the values were almost identical. Interestingly, the (35–41>) age range had the lowest β-hCG MoM values in the study group, while in the control group, the lowest values were obtained in the (30–35>) age range Figure 5.

Table 4 Basic Statistics of PAPP-A MoM

Group	Group	MoM	Minimum	Maximum	Standard Deviation
Study group	105	1.099	0.13	3.089	0.625
Control	105	1.036	0.19	3.43	0.587

Notes: Study group – women diagnosed with hypothyroidism before or during pregnancy. Control group – women of a similar body weight and gestational age without hypothyroidism.

Table 5 Results of Student's t-test for the Two Mean PAPP-A MoM Values

	Sample	t	df	p	p Variance
PAPP-A MoM	105	0.754	208	0.452	0.517

Notes: t – Student's t-distribution. df – number of degrees of freedom. *p* – (*p*-value) test probability. *p* variance – (*p*-variance) – test probability for variance (Levene's test).

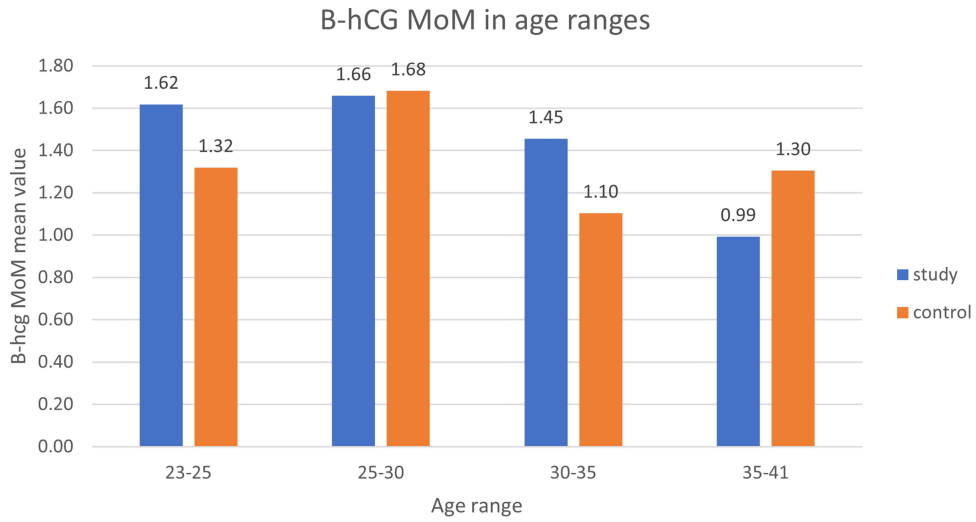

Figure 4 Bar graphs showing the effect of age on β-hCG in the study and control groups.

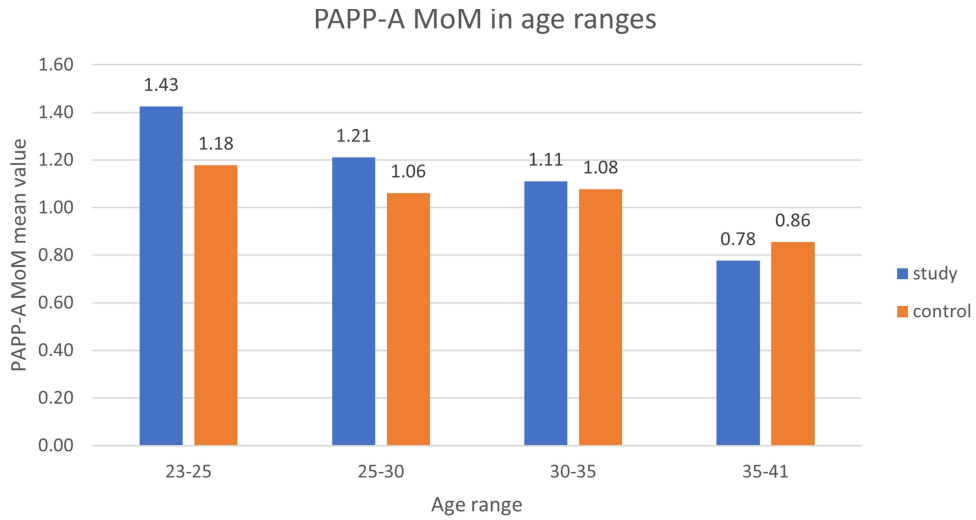

Figure 5 Bar graphs showing the effect of age on the PAPP-A MoM in the study and control groups.

Noteworthy is that the mean PAPP-A MoM decreased along with increasing age. Again, the eldest women in the study group (35–41>) had the lowest value of this parameter. The remaining age groups were characterized by higher mean values in the study group. The greatest difference could be observed in the youngest women Figure 6.

In most of the weight ranges adopted, there were clear differences in the β-hCG MoM values, especially in women with a higher body weight (over 70 kg). In the 60–70 kg range, the values for both groups were almost identical. It should be noted that almost 50% of study participants from both groups were in this weight range. Considering only women with a higher body weight, one could conclude that hypothyroidism increases the β-hCG MoM. The results obtained in the 60–70 kg weight range did not confirm it, but also did not allow concluding that hypothyroidism does not affect the pregnancy parameters tested. It is worth noting, however, that the β-hCG MoM value decreased along with the increasing BMI of pregnant women Figure 7.

For the PAPP-A MoM, the results were ambiguous as well. In one half of the weight ranges adopted, the mean PAPP-A MoM was greater in the study group, and in the other half of the weight ranges, a higher value was obtained in the control group. In addition, differences between the two groups were not high, ranging from 0.03 to 0.16. Only in women

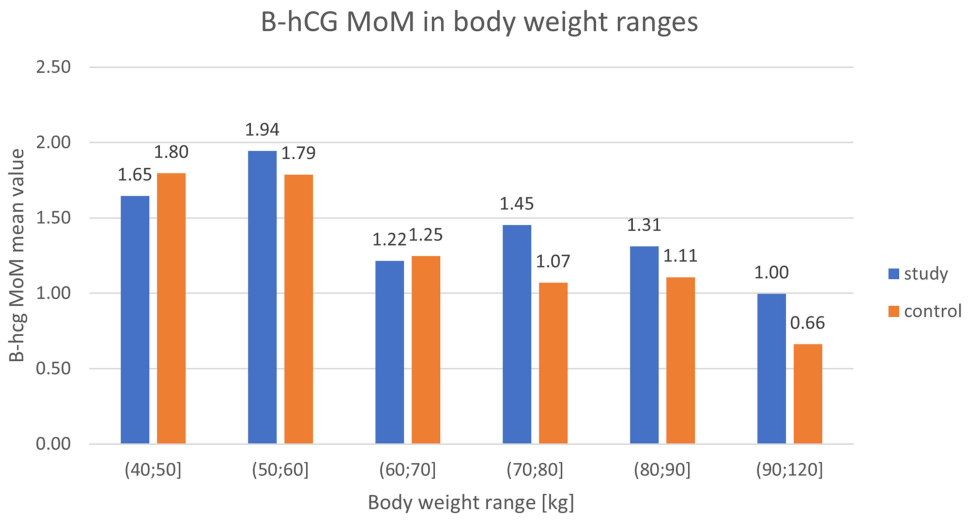

Figure 6 Mean values of the β-hCG MoM by body weight range.

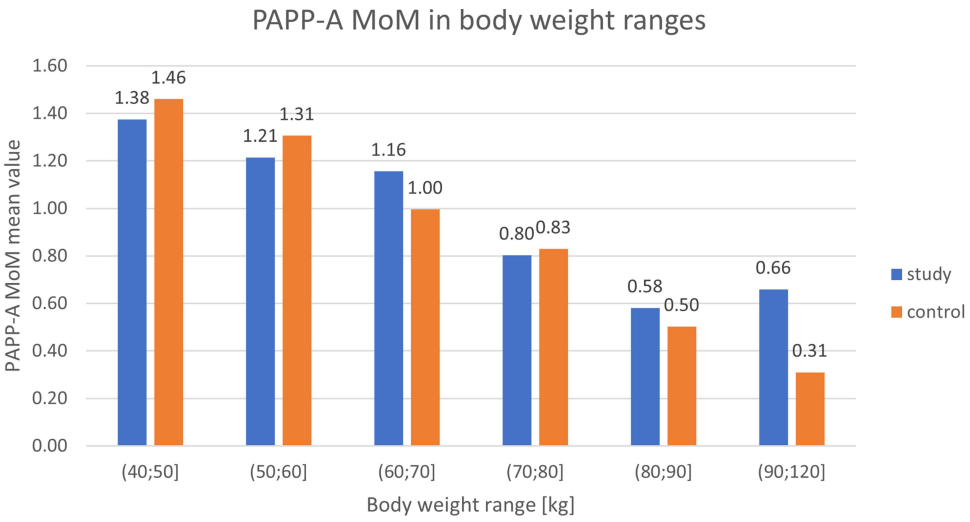

Figure 7 Mean values of the PAPP-A MoM by body weight range.

weighing more than 90 kg, the difference was pronounced, but this weight range was not represented by a number of women high enough to draw clear conclusions.

There was no noticeable impact of hypothyroidism on the change in the PAPP-A MoM depending on body weight. This parameter was linearly correlated with the weight of the study participants – the higher the BMI, the lower the MoM of the PAPP-A concentration. This correlation is associated with the so-called dilution effect. The high body weight of a pregnant woman affects the concentrations of the parameters tested, thus reducing their levels by dilution, and the substance is distributed over a larger vessel capacity.

Discussion

The mean values of PAPP-A MoM and β-hCG MoM were compared in the group of women with hypothyroidism diagnosed before or during pregnancy and in the group of women of a similar body weight and gestational age without hypothyroidism. The difference in mean levels between the study group and the control group was 0.063 for PAPP-A and 0.075 for β-hCG. Patients with hypothyroidism had higher β-hCG MoM and PAPP-A MoM values. Interestingly, there

was a higher number of patients with outlier values in the study group (9) than in the control group (5). Additional factors, such as age and body weight, and their effect on the parameters examined, were analysed as well.

β-hCG is a glycoprotein secreted by the syncytiotrophoblast (ST) into both the fetal and the maternal circulation, and it is structurally similar to TSH and luteinizing hormone. The most well-known biological function of β-hCG is to stimulate the corpus luteum to produce progesterone. High β-hCG concentrations in early pregnancy are believed to directly activate the TSH receptor.[15–17] Glinoer et al reported that β-hCG concentration in early pregnancy is negatively correlated with TSH concentration in blood serum and positively correlated with free T4 concentration.[18] Some studies have suggested that thyroid hormones affect the secretion of placental hormones, including β-hCG.[19,20] Maruo et al studied the direct influence of T3 and T4 on the function of trophoblasts using human placenta tissue culture. They demonstrated that the increased amount of T3 and T4 results in a maximum increase in the daily secretion of progesterone, estradiol, β-hCG and α-hCG by the placenta tissue cultured. However, higher levels of T3 and T4 within this tissue did not increase the endocrine activity itself.[21] In numerous studies conducted by Kagan et al, high levels of β-hCG were detected in trisomy 21, and low in trisomies 13 and 18. Similar observations were made by other groups.[22] PAPP-A is produced by the ST and is the largest pregnancy-associated glycoprotein.[23] PAPP-A is a metalloproteinase that cleaves insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and is an important regulator of IGF bioavailability and cell growth. PAPP-A concentration in the blood of pregnant women is often reduced in fetal aneuploidy, and low concentration may be associated with intrauterine growth restriction, premature birth, pre-eclampsia and placental abruption.[24] Decrease PAPP-A concentration suggests abnormal production and function of the ST. In normal pregnancy, there is placental transport of free thyroxine (fT4) which is transformed intracellularly into triiodothyronine (T3). Maruo et al demonstrated that T3 and T4 increase progesterone secretion.[13,21]

A study by Lawrence et al showed that IGFBP-4 is a strong inhibitor of IGF in vitro, and the cleavage of IGFBP-4 eliminates its inhibition of the stimulatory effect of IGF on various systems. This suggests that IGFBP-4 proteolysis functions as a positive regulator of IGF bioavailability. PAPP-A has been identified as an IGF-dependent IGFBP-4 protease.[25] According to Näntö-Salonen et al, thyroid hormones regulate IGF and the expression of IGFBP.[26]

According to Aytan et al, there is no link between the function of the maternal thyroid and biochemical markers in the first trimester of pregnancy, and no statistically significant correlation was found between TSH in pregnant women and the concentrations of hCG and pregnancy-associated plasma protein.[27] Also, studies by Erol et al did not confirm significant statistical differences in the results of prenatal non-invasive screening tests. However, it has been shown that pregnancies complicated by the presence of thyroid autoantibodies require more caution because of the potential for adverse pregnancy outcomes for the mother and the fetus. Therefore, as the authors emphasize, monitoring the maternal thyroid status and performing functional tests is important during pregnancy.[28]

Hantoushzadeh et al showed an association between thyroxine and β-hCG, and considered that assessing hormones such as T4 may affect the interpretation of screening tests for pregnancy pathologies, especially in pregnancies with chromosomal aberrations.[29]

Our study analysed a relatively small group of pregnant women. Increasing the size of the study group and the ability to assess further stages of pregnancy and the postnatal condition of the newborn would have allowed drawing broader conclusions.

Notably, most patients during the study received thyroid hormone replacement therapy. Therefore, the results obtained may not be conclusive. The study group included patients with hypothyroidism occurring before pregnancy and patients in whom hypothyroidism was diagnosed during pregnancy. Hormone replacement during the periconceptional period and in the 1st trimester of pregnancy may have resulted in the small difference in the obtained values of the parameters examined. Inclusion of only those women who received therapy during pregnancy may have produced more pronounced differences.

The lower β-hCG values obtained in women of the study group over 35 years of age, compared with the control group, suggest that the outcome of genetic risk assessment is most distorted in this group. Therefore, conducting such an assessment should not be recommended in this group.

Conclusions

1. Pregnant women with hypothyroidism have higher median values of the plasma concentrations of PAPP-A and the free subunit of β-hCG than pregnant women without hypothyroidism, which may affect the assessment of genetic risk.
2. Introducing correction for patients with hypothyroidism during non-invasive biochemical prenatal testing may allow obtaining more reliable results that would be the basis for referral to invasive procedures.
3. The most unreliable non-invasive test results in women with hypothyroidism were obtained in the group over 35 years of age. Therefore, these patients should be offered a higher sensitivity test.

Ethics Approval and Consent to Participate

The study was performed in accordance with the Declaration of Helsinki and accepted standards of ethics. The consent of the relevant bioethics committee was obtained: Bioethics Committee of Nicolaus Copernicus University, Toruń, Poland, N^{o} 551/2019.

Consent to Publish

All patients signed written informed consent for the publication of their clinical data.

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Disclosure

The authors declare that they have no competing interests for this work.

References

1. Hubalewska-Dydejczyk A, Lewiński A, Milewicz A, et al. Management of thyroid diseases during pregnancy. *Endokrynol Pol*. 2011;62:362–381.
2. Krysiak R, Okopień B, Szkróbka W, et al. Thyroid disorders in pregnancy and after delivery. *Przegl Lek*. 2007;64:159–164.
3. Parkes IL, Schenker JG, Shufaro Y. Thyroid disorders during pregnancy. *Gynecol Endocrinol*. 2012;28:993–998. doi:10.3109/09513590.2012.692001
4. Gietka-Czernel M, Glinicki P. Subclinical hypothyroidism in pregnancy: controversies on diagnosis and treatment. *Pol Arch Intern Med*. 2021;131:266–275. doi:10.20452/pamw.15626
5. Kucharska AM, Beń-Skowronek I, Walczak M. The treatment and monitoring of the therapy of congenital hypothyroidism. *Pediatr Endocrinol Diabetes Metab*. 2016;21:127–131. doi:10.18544/PEDM-21.03.0034
6. Sullivan SA. Hypothyroidism in pregnancy. *Clin Obstet Gynecol*. 2019;62:308–319. doi:10.1097/GRF.0000000000000432
7. Dong AC, Morgan J, Kane M, Stagnaro-Green A, Stephenson MD. Subclinical hypothyroidism and thyroid autoimmunity in recurrent pregnancy loss: a systematic review and meta-analysis. *Fertil Steril*. 2020;113:587–600. doi:10.1016/j.fertnstert.2019.11.003
8. Menif O, Omar S, Feki M, et al. Hypothyroidism and pregnancy: impact on mother and child health. *Ann Biol Clin*. 2008;66:43–51.
9. Zhang Y, Wang H, Pan X, et al. Patients with subclinical hypothyroidism before 20 weeks of pregnancy have a higher risk of miscarriage: a systematic review and meta-analysis. *PLoS One*. 2017;12:e0175708. doi:10.1371/journal.pone.0175708
10. Ouzounian S, Bringer-Deutsch S, Jablonski C, et al. Hypothyroidism: from the desire for pregnancy to delivery. *Adv Clin Exp Med*. 2016;25:457–463. doi:10.17219/acem/38555
11. Adamarczuk-Janczyszyn M, Zdrojowy-Wełna A, Rogala N, et al. Evaluation of selected atherosclerosis risk factors in women with subclinical hypothyroidism treated with L-thyroxine. *Adv Clin Exp Med*. 2016;25:457–463.
12. Lee SY, Pearce EN. Testing, monitoring, and treatment of thyroid dysfunction in pregnancy. *J Clin Endocrinol Metab*. 2021;106:883–892. doi:10.1210/clinem/dgaa945
13. Nikolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol*. 2004;191:45–67. doi:10.1016/j.ajog.2004.03.090
14. Nikolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn*. 2011;31:7–15. doi:10.1002/pd.2637
15. Hörmansdörfer C, Schmidt P, Hillemanns P, et al. The prenatal detection of trisomy 13, 18, and 21: comparison of the advanced first trimester screening (AFS) with the first trimester screening according to Nicolaides. *Z Geburtshilfe Neonatol*. 2007;211:243–249. doi:10.1055/s-2007-981361
16. Newby D, Aitken DA, Howatson AG, Connor JM. Placental synthesis of oestriol in Down's syndrome pregnancies. *Placenta*. 2000;21:263–267. doi:10.1053/plac.1999.0469
17. Ardawi MS, Nasrat H, Rouzi A, et al. Maternal serum free-beta-chorionic gonadotrophin, pregnancy-associated plasma protein-A and fetal nuchal translucency thickness at 10-13(+6) weeks in relation to co-variables in pregnant Saudi women. *Prenat Diagn*. 2007;27:303–311. doi:10.1002/pd.1661
18. Glinoer D, de Nayer P, Bourdoux P, et al. Regulation of maternal thyroid during pregnancy. *J Clin Endocrinol Metab*. 1990;71:276–287. doi:10.1210/jcem-71-2-276
19. Sieroszewski P, Słowakiewicz K, Perenc M. Interpretation of false positive results of biochemical prenatal tests. *Ginekol Pol*. 2010;81:210–214.

20. Korevaar T, Rich Y, Chaker L, et al. Stimulation of thyroid function by human chorionic gonadotropin during pregnancy: a risk factor for thyroid disease and a mechanism for known risk factors. *Thyroid*. 2017;27:440–450. doi:10.1089/thy.2016.0527
21. Maruo T, Matsuo H, Mochizuki M. Thyroid hormone as a biological amplifier of differentiated trophoblast function in early pregnancy. *Acta Endocrinol*. 1991;125:58–66. doi:10.1530/acta.0.1250058
22. Kagan KO, Sonek J, Kozlowski P. Antenatal screening for chromosomal abnormalities. *Arch Gynecol Obstet*. 2022;30:825–835. doi:10.1007/s00404-022-06477-5
23. Smith GC, Stenhouse EJ, Crossley JA, et al. Early pregnancy levels of pregnancy associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab*. 2002;87:1762–1767. doi:10.1210/jcem.87.4.8430
24. Przybylski G, Pasińska M, Pyskir J, et al. Analysis of spreading of smoking habit among pregnant women admitted to the Prenatal Outpatient Clinic in 2005–2006. *Przeglad lekarski*. 2007;64:827–830.
25. Lawrence JB, Oxvig C, Overgaard MT, et al. The insulin - like growth factor (IGF) - dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci USA*. 1990;96:3149–3153. doi:10.1073/pnas.96.6.3149
26. Näntö-Salonen K, Muller HL, Hoffman AR, et al. Mechanisms of thyroid hormone action on the insulinlike growth factor system: all thyroid hormone effects are not growth hormone mediated. *Endocrinology*. 1993;132:781–788. doi:10.1210/endo.132.2.7678799
27. Aytan H, Caliskan AC, Demirturk F, et al. Relationship between maternal thyroid hormones and the biochemical markers of the first trimester aneuploidy screening. *Arch Gynecol Obstet*. 2013;287:1125–1129. doi:10.1007/s00404-013-2712-4
28. Erol SA, Caglar AT, Engin Ustun Y, et al. Evaluation of perinatal and neonatal outcomes in pregnant women with thyroid autoantibody positivity (anti-thyroglobulin and anti-thyroid peroxidase) due to thyroiditis. *SN Compr Clin Med*. 2022;4:69–72. doi:10.1007/s42399-022-01151-y
29. Hantoushzadeh S, Tara F, Salmanian B, et al. Correlation of nuchal translucency and thyroxine at 11–13 weeks of gestation. *J Matern Fetal Neonatal Med*. 2013;26:1586–1589. doi:10.3109/14767058.2013.784259