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ORIGINAL ARTICLE

Maternal fT4 blood level during pregnancy in euthyroid pregnant women correlates positively with neonate's Apgar score

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Abstract

OBJECTIVES: The study investigated, whether the FT4 and TSH blood levels in euthyroid pregnant women correlate with neonatal Apgar score.
MATERIAL AND METHODS: The study group included 102 euthyroid pregnant women and their newborns. All women underwent clinical assessment (including pregnancy and perinatal interview and physical examination) and laboratory tests involving TSH and FT4 blood concentrations measurements three times, in each trimester. The neonate's overall condition was assessed with the Apgar scale.
RESULTS: A significant positive correlation was described between FT4 concentrations in the study group in the first and second trimester and the newborns' condition assessed on the Apgar scale at 1 minute (Spearman's Rs rank correlation, R = 0.2418; $p = 0.014$ for the first trimester, and R = 0.2015; $p = 0.043$ for the second trimester); FT4 concentrations in the first trimester and Apgar score at 3 minutes (Spearman's Rs rank correlation, R = 0.2066; $p = 0.038$) and between FT4 in the second trimester and the results of Apgar score in the 5th minute (Spearman's Rs rank correlation, R = 0.2231; $p = 0.024$). Otherwise, maternal TSH concentrations did not significantly correlate with neonatal Apgar score. Analysis of the correlation between the tested thyroid hormones and the birth weight of newborns, as well as delivery date, did not show any significant correlations
CONCLUSION: Our study disclosed the impact of maternal FT4 concentration on neonatal outcome expressed by Apgar score. The results presented reveal potential clinically relevant benefits derived from profound thyroid screening during pregnancy.

INTRODUCTION

Numerous adaptive changes are happening in a woman's body during pregnancy. The endocrine system, including the thyroid and its hormones, plays a significant role in these adjustments and the pituitary-thyroid axis also undergoes specific rearrangements. The concentrations of free thyroxine (fT4) essential for proper body function during pregnancy increase significantly. The elevation of maternal estrogen levels, leading to the increase of thyroxine-binding globulin (TBG) production, and the degrading activity of placental deiodinase type 3 on thyroid hormones, both cause a functional inactivation of a significant part of thyroid hormones. In response, the regulation of TSH (thyroid-stimulating hormone) is partially driven by the high levels of human chorionic gonadotropin (hCG) reaching its maximum at the end of the first trimester. The weak thyroid stimulating activity of hCG, similar to TSH, causes the decrease of TSH blood concentrations and increase of fT4 levels (Springer *et al.* 2017; Mégier *et al.* 2023; Aref *et al.* 2024). Such advanced changes enabling proper maternal thyroid function are needed both for the mother and the developing fetus. Thyroid hormones are essential for the physiological growth and maturation of cells during gestation. The abnormal functioning of the maternal thyroid may lead to the dysregulation of metabolism, neuromuscular conduction, and the impaired development of the nervous and bone tissue (Springer *et al.* 2017; Mégier *et al.* 2023; Aref *et al.* 2024).

Even around 10% of pregnant women may present with some kind of thyroid dysfunction, and the most prevalent are subclinical hypothyroidism, overt hypothyroidism, and subclinical hyperthyroidism (Gupta *et al.* 2021; Mahadik *et al.* 2020). Subclinical hypothyroidism is a condition where increased levels of TSH are accompanied by fT4 within the normal range. While in some countries (like Poland, where our research was conducted) universal screening of thyroid function is recommended, it remains controversial, since most dysfunctions are mild and asymptomatic (Taylor *et al.* 2018). 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum state that there is no clear evidence for or against universal screening of TSH levels in early pregnancy. Moreover, Guidelines… do not recommend universal screening to detect low fT4 concentrations, however, the strength of that recommendation is weak and it is based on moderate-quality evidence (Alexander *et al.* 2017).

Many laboratory tests can be used to assess thyroid function and the concentrations of related hormones (Visser & Peeters 2020; Fitzgerald *et al.* 2022). From the physiological and observational point of view, studies suggest that fT4 levels might be more indicative than TSH of the actual thyroid state of both mother and fetus. While the placenta does not allow passage of TSH, there is evidence of T4 crossing the placental barrier in the early stages of pregnancy (Fitzgerald *et al.* 2022; Contempré *et al.* 1993). Moreover, in cases of subclinical hyperthyroidism, there are no adverse pregnancy risks associated that can be observed in clinical hyperthyroidism (Casey *et al.* 2006). However, the assay of fT4 is particularly challenging, since only about 0,03% of the total T4 is presented in the serum in its unbound form. Hence, there are many controversies that the reliability of the fT4 assessment might be burdened with significant exogenous and endogenous interference (Visser & Peeters 2020; Fitzgerald *et al.* 2022).

Our study aimed to assess the relationship between blood fT4 levels in healthy women during pregnancy and a newborn's health condition measured by the Apgar score.

MATERIAL AND METHODS

Study group

The study was designed prospectively. The study group included 102 pregnant female patients and their newborns. The inclusion criteria were: the patient's age (18-40 years old), the absence of relevant comorbidities described in the exclusion criteria, and informed consent to the study participation. Patients with psychiatric disorders reported, with chronic diseases influencing their mental and hormonal balance, with endocrinological disorders before pregnancy or under hormonal treatment were excluded from the study.

The study's project was approved by the Poznań University of Medical Sciences Bioethics Committee. All women enrolled in the study were informed about the aim of the research and the types of tests and all signed the informed consent.

The examinations of the pregnant women from the study group were performed at the 12th, 24th, and 36th weeks of the pregnancy and included clinical and laboratory tests. Clinical assessment included pregnancy and perinatal medical interview and physical examination. Besides, body mass index (BMI) was measured twice – at the first and last examination included in the study.

The laboratory tests involved the measurement of the free thyroxine (fT4) and TSH blood levels. The blood samples were taken in the morning (8:00), fasting. To assess the fT4 and TSH levels in the blood sample, the enzyme-linked immunosorbent assay (ELISA kit, Labor Diagnostika Nord) was used. The diagnostic test sensitivity amounts to 1,0 pg/ml for fT4 and 0,06 mIU/l-1 for TSH.

The newborn's overall condition was assessed with the Apgar score. The examination was performed by a pediatrician at the 1st, 3rd, and 5th minute of the neonate's life. The Apgar scoring system is a test with approved utility, commonly performed to assess the general state of a newborn. It was created by Dr Virginia

Apgar in 1952 and it consists of 5 parameters: muscle tone, heart rate, response to stimulation (such as suction of the baby's nose), color, and breathing. In every category 0, 1, or 2 points can be granted. Furthermore, all neonates were weighed, after blotting, without clothes, within 10 minutes of labor and before the first feeding. The measurements were taken with an electronic scale and the results were given in grams.

Moreover, the umbilical cord blood pH and base excess (BE) were measured. The blood in the amount of 2 ml was sampled from the umbilical cord artery right after clenching the umbilical cord to the syringe containing heparin. The gasometry was assessed within 30 minutes after the samples were taken. The analysis was performed in the usage of the COBAS B221 machine.

Statistical analysis

The statistical analysis was performed using Statistica 12 and StatXact programs, with the significance level $\alpha = 0.05$. The following tests were used: Fisher-Freeman-Halton test for categorical variables for correlation assessment; Shapiro-Wilk test for interval scale variables for checking the compliance with the normal distribution; Mann-Whitney test in case of disagreement with normal distribution; t-Student test for independent samples for variables with normal distribution and equal variances. When comparing 3 or more groups of variables compliant with normal distribution and equal variances, the analysis of variance test for independent samples was used. In case of non-compliance with normal distribution, the Kruskal-Wallis test was calculated. If statistically significant differences were found, Dunn's post-hoc test with multiple comparisons was calculated. To examine the relationship between continuous variables, in the non-compliance with the normal distribution, the rank correlation coefficient Rs Spearman was calculated. When the variables were compliant with the normal distribution, the linear correlation coefficient r Pearson was assessed. To check whether the changes during pregnancy are statistically significant, for parameters in the absence of compliance with normal distribution or variables measured on an ordinal scale, a Friedman test was calculated. If significant differences were found, to assess which points in time differ, Dunn's multiple comparisons post-hoc test was used.

RESULTS

Pregnant women

Demographic and socioeconomic characteristics

A total of 102 pregnant female patients were enrolled in the study. The median age in the study group was 30.4 ± 4.4 years (ranging from 19 to 40 years). The majority of participants lived in a city (80 patients; 78.4%) and did not work during pregnancy (70 patients; 68.6%). Moreover, almost half of the study group (48 participants; 47%) received higher education. The vast majority declared not to take any stimulants during pregnancy (97 patients; 95%). Details are shown in Table 1.

Tab. 1. Demographic, socioeconomic and clinical characteristics of pregnant women from the study group

Characteristics	Study group (N = 102)
Age – mean ± SD [years]	30.4 ± 4.4
Residence – no. (%)	
Rural	22 (21.6%)
Urban	80 (78.4%)
Education – no. (%)	
<12 years	16 (16%)
12 years	38 (37%)
>12 years	48 (47%)
Work during pregnancy – no. (%)	
No	70 (68.6%)
Yes, only I trimester	21 (20.5%)
Yes, I and II trimesters	7 (6.8%)
Yes, whole pregnancy	4 (4.0%)
Stimulants – no. (%)	
No	97 (95%)
Tobacco	4 (3.9%)
Alcohol	1 (0.98%)
Drugs	0 (0%)
BMI – mean ± SD [kg]	
I trimester	24.15 ± 5.4
III trimester	29.03 ± 5.2

Clinical characteristics

As given in the Material and Methods section, the study group consisted of healthy pregnant women with the absence of relevant comorbidities described in the exclusion criteria.

Hormonal profile

The hormonal profile was assessed in the study group. The cortisol, prolactin, progesterone, and estradiol levels increased significantly in subsequent trimesters of pregnancy ($p < 0.05$). On the contrary, fT4 concentration decreased in subsequent trimesters of pregnancy, with statistically significant differences observed between the first and third trimesters ($p < 0.05$). No significant changes in TSH concentration were observed in the study group during pregnancy.

Newborns

All the newborns from the study group were born between 36 and 41 weeks of gestation, with an average

Tab. 2. Clinical characteristics of newborns

Characteristics	Study group (N = 102)
Gestational age – mean ± SD [weeks]	38.1 ± 0.82
Type of birth – no. (%)	
Natural	76 (74.5%)
Cesarean section	26 (25.4%)
Sex – no. (%)	
Female	50 (49%)
Male	52 (51%)
Birth weight – mean ± SD [kg]	3328.7 ± 337.1
Apgar score at 1 min – no. (%)	
10	77 (75.4%)
9	10 (9.8%)
8	4 (3.9%)
7	6 (5.8%)
6	2 (1.9%)
5	1 (0.98%)
4	0 (0%)
3	1 (0.98%)
Apgar score at 1 min – mean ± SD	9.4 ± 1.2
Apgar score at 3 min – no. (%)	
10	88 (85.3%)
9	7 (6.8%)
8	4 (3.9%)
7	3 (2.9%)
6	0 (0.0%)
5	0 (0.0%)
4	0 (0.0%)
3	0 (0.0%)
Apgar score at 3 min – mean ± SD	9.7 ± 0.6
Apgar score at 5 min – no. (%)	
10	97 (95.1%)
9	3 (2.9%)
8	2 (1.9%)
7	0 (0.0%)
6	0 (0.0%)
5	0 (0.0%)
4	0 (0.0%)
3	0 (0.0%)
Apgar score at 5 min – mean ± SD	9.9 ± 0.3
Umbilical cord blood pH – mean ± SD	7.3 ± 0.08
BE – mean ± SD	-3.1 ± 2.9

gestational age of 38.1 ± 0.8 weeks. Preterm birth (in the 36th week of gestation) concerned only 2 newborns (1.96%) and they remained included in the study as it did not influence significantly the consistency of the study group. The vast majority (74.5%) were born naturally, whereas the remaining 25.5% were born by a cesarean section. The mean birth weight of newborns was 3328.7 ± 337.1. and was within the normal range (in Poland: 2500-4000 g) for all full-term newborns. 50 (49%) of the children born were female, and 52 (51%) were male. The average Apgar in newborns was 9.4 ± 1.2 in 1st minute, 9.7 ± 0.6 in 3rd minute, and 9.9 ± 0.3 in 5th minute. The average pH of umbilical cord blood was 7.3 ± 0.08, and the BE value was -3.1 ± 2.9. For details, see Table 2.

Correlations between maternal thyroid profile and newborn's condition

Interestingly, a significant positive correlation was described between fT4 concentrations in the study group in the first and second trimester and the newborns' condition assessed on the Apgar scale at 1st minute (Spearman's Rs rank correlation, R = 0.2418; p = 0.014 for the first trimester, and R = 0.2015; p = 0.043 for the second trimester; Figures 1 and 2).

A significant correlation was also observed between fT4 concentrations in the first trimester and Apgar score at 3rd minute (Figure 3). Higher concentrations of fT4 were associated with higher Apgar at 3rd minute (Spearman's Rs rank correlation, R = 0.2066; p = 0.038). A significant positive correlation was also found between fT4 in the second trimester of pregnancy and the results of the Apgar score in the 5th minute (Spearman's Rs rank correlation, R = 0.2231; p = 0.024; Figure 4). There was no statistically significant correlation between maternal TSH blood level and neonate's Apgar score.

Statistical analysis showed a single, statistically significant correlation between umbilical cord blood pH and fT4 in the second trimester of pregnancy. The higher the concentration of fT4, the higher the umbilical cord blood pH (Spearman's Rs rank correlation, R = 0.2489; p = 0.011). Statistical analysis did not reveal any significant relationship between the concentration of hormones and BE umbilical cord blood.

Analysis of the correlation between the tested thyroid hormones and the birth weight of newborns, as well as delivery date, did not show any significant correlations ($p > 0.05$).

DISCUSSION

The proper function of the maternal thyroid is crucial for fetal development and healthy pregnancy. Besides fetal neurodevelopment, results reveal that maternal thyroid function influences fetal growth. Shields *et al.* in the results of their study indicate a significant positive correlation between neonatal birthweight and fT4 level

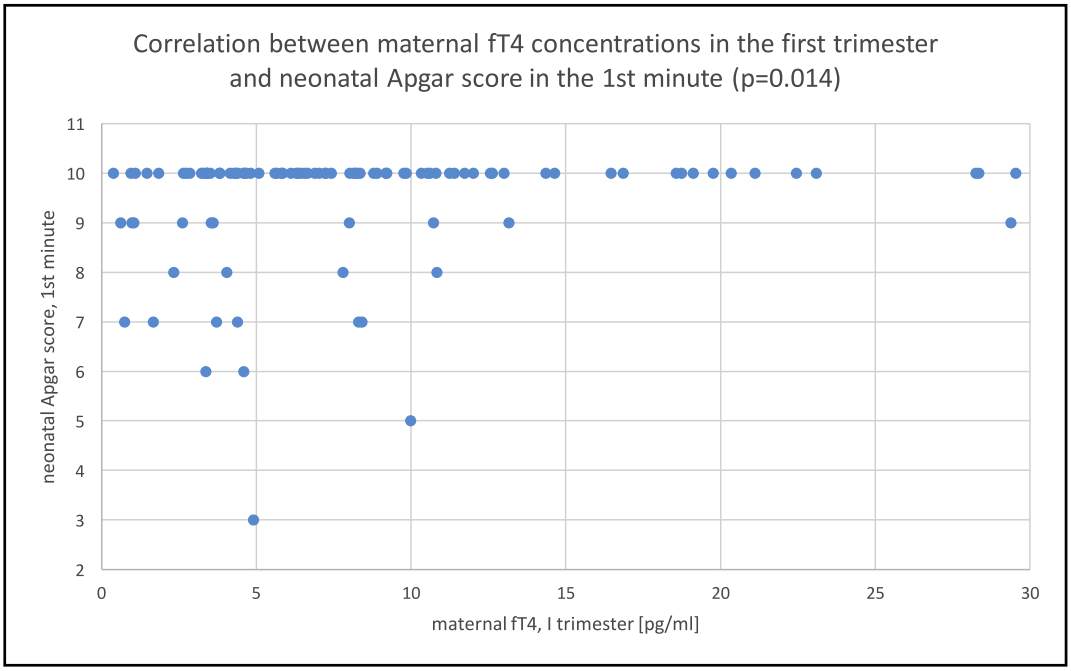

Fig. 1. Correlation between maternal fT4 concentrations in the first trimester and neonatal Apgar score in the 1st minute (p = 0.014)

in umbilical cord blood. Moreover, fT4 umbilical cord blood level was positively correlated with fT4 maternal level and such an association was not shown concerning TSH (Shields *et al.* 2011). Results of the study conducted on 3945 pregnant women in China point out the statistically significantly higher incidence of preterm birth, premature rupture of membranes, and low Apgar score at the first minute after birth in a group of women with isolated hypothyroxinemia in the third trimester (Chen *et al.* 2023). On the other hand, an American study of 17298 pregnant women examining the association between maternal isolated hypothyroxinemia and adverse neonatal outcomes showed no significant correlation, aside from the possible parallel between maternal hypothyroxinemia and preterm birth (Casey *et al.* 2007). Another study enrolling 1055 pregnant women indicates significant adverse effects on maternal and fetal outcomes associated with thyroid dysfunction, especially overt and subclinical hypothyroidism among Indian pregnant women, along with lower neonate's Apgar score, respiratory distress, or preterm delivery (Prabhat *et al.* 2023). Taking all the mentioned research

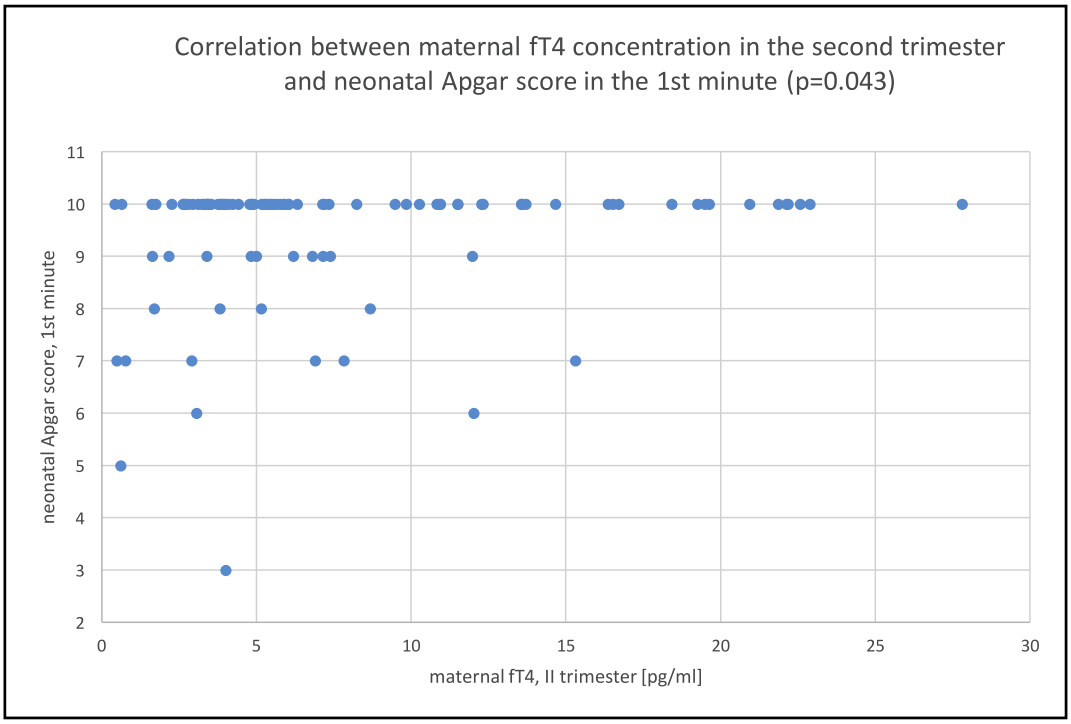

Fig. 2. Correlation between maternal fT4 concentrations in the second trimester and neonatal Apgar score in the 1st minute (p = 0.043)

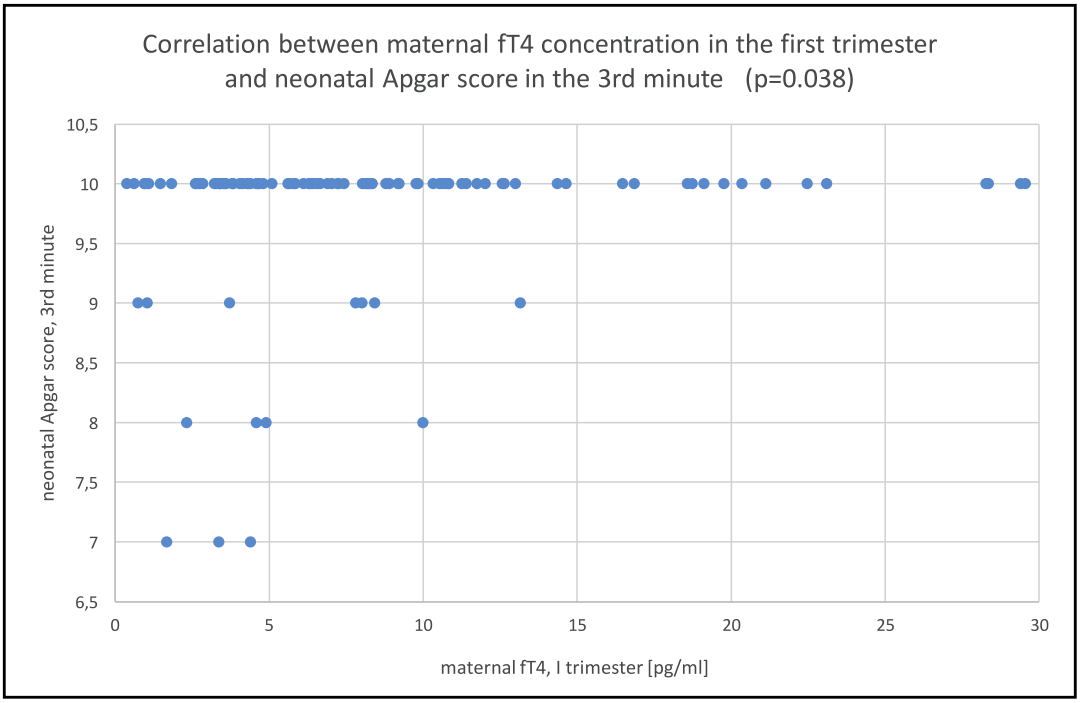

Fig. 3. Correlation between maternal fT4 concentrations in the first trimester and neonatal Apgar score in the 3rd minute (p = 0.038)

and results of our study into consideration, the exact association between maternal hypothyroxinemia and adverse pregnancy outcomes remains unclear, but the negative consequences of maternal thyroid dysfunction both on pregnant women and newborns should not be diminished. Interesting conclusions were presented, based on the results of the Avramowska *et al.* study, that total thyroxine and thyroglobulin can be used as determinants for predicting the neonatal outcome, expressed through birth weight and Apgar score (Avramovska *et al.* 2021). What cannot be omitted is the fact, that our study involved pregnant women without any endocrine disorders, which indicates the auspicious benefits deriving from enhanced healthcare attention to maternal thyroid profile during pregnancy. The possible management of low fT4 blood level and its undesirable consequences seems to be levothyroxine supplementation. There is a recent study investigating the effect of extra-low dose levothyroxine supplementation on pregnancy outcomes in women with subclinical hypothyroidism undergoing in vitro fertilization and embryo transfer (Chen *et al.* 2023). According to its

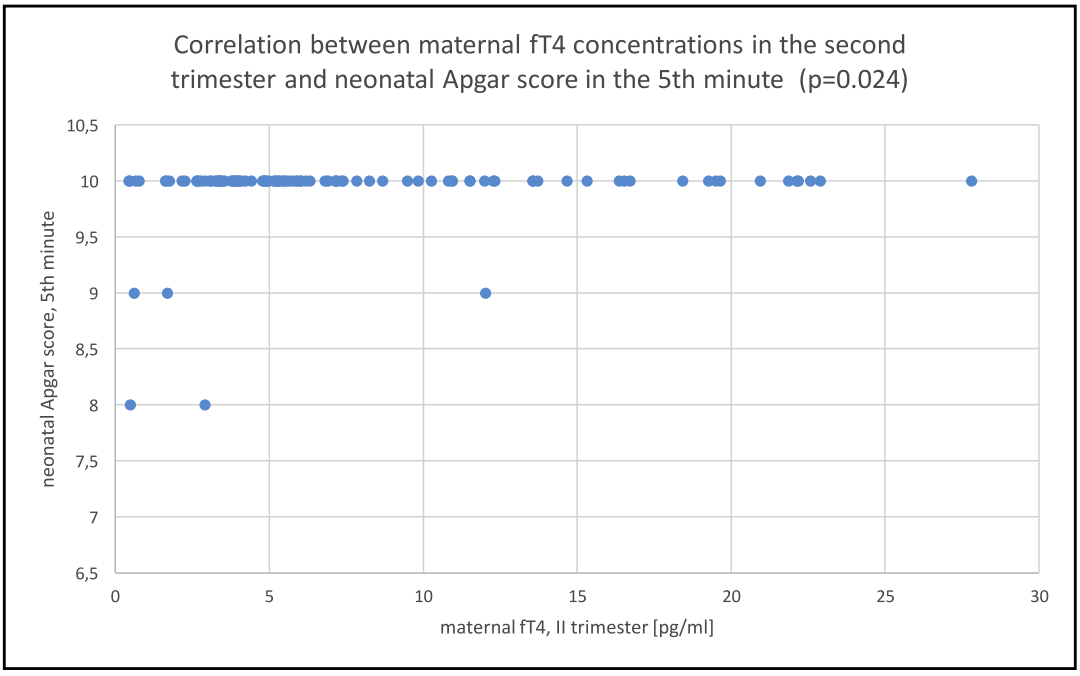

Fig. 4. Correlation between maternal fT4 concentrations in the second trimester and neonatal Apgar score in the 5th minute (p = 0.024)

results, even an extra-low dose of levothyroxine used in the treatment of subclinical hypothyroidism during pregnancy led to comparable obstetrical and neonatal outcomes as that in euthyroid pregnant women (Chen *et al.* 2023). Looking at both just mentioned and our study results, the levothyroxine supplementation seems to give promising results, but the question remaining is which fT4 blood level requires such handling.

However, it is still hard to explicitly point out whether a thyroid status test should become a routine screening during pregnancy. There are two main strategies regarding thyroid screening in pregnancy implemented in most countries: the case-finding approach and universal screening. The first method means the restriction of screening to women who are, by specific criteria, perceived as high-risk. It is recommended by major organizations, such as ATA, ACOG (The American College of Obstetricians and Gynecologists), and SMFM (Society for Maternal-Fetal Medicine) (Alexander *et al.* 2017; American College of Obstetricians and Gynecologists 2020; Chang & Pearce 2013). While the definitions of 'high-risk' women presented in different guidelines vary considerably, it appears to be unanimous to recommend testing for TSH over fT4 as the primary test (Alexander *et al.* 2017; American College of Obstetricians and Gynecologists 2020; Chang & Pearce 2013). Alternatively, the universal approach is not subject to the opinion of who is and who is not considered for screening. It is also much more sensitive and can detect more women with subclinical conditions, however, it remains controversial if such minute abnormalities are worth managing from both therapeutic and economic points of view (Amiri *et al.* 2022).

The idea of pregnancy thyroid screening is mostly focused on identifying undiagnosed conditions. Our results show that healthy pregnant women may also benefit from the thyroid evaluation. Moreover, even though TSH is generally recommended across different guidelines, it is not free of limitations and interpretation difficulties (Karavani *et al.* 2022). Additional tests, such as fT4 concentrations, seem to broaden the extent of patients who might need further medical attention. Combined with previously mentioned research, our work shows that there is a need to extend or re-think thyroid screening strategies.

The results of our research presented above point out the positive correlation between maternal fT4 blood level and neonate's outcome expressed in Apgar score. To our knowledge, it is the first study revealing such a parallel in the group of pregnant female patients with no previously diagnosed, treated, or existing thyroid dysfunction.

Pregnancy and perinatal healthcare remain one of the principal objectives for the general healthcare system, so we would like to emphasize the necessity of constant improvement of prophylaxis in the field of thyroid profile abnormalities during pregnancy. We hope that the results of this study will add a new perspective to the discussion.

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