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Diabetes and Hypothyroidism Alone and Simultaneously in Bulgarian Pregnant Women - Frequency and Features of Various Risk Factors

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Abstract: Gestational hypothyroidism affects the maturation and function of the beta cell, which can influence glucose metabolism. *The aim* of the study is to investigate the relationship between Hypothyroidism and Hyperglycemia in Bulgarian pregnant women and to look for the influence of various factors on the manifestation of each of these diseases separately, as well as their role in cases of simultaneous combination of the two diseases. *Material*: We studied 547 pregnant women, mean 30±5 years. The cross-sectional population-based multicenter study was conducted in 84 Bulgarian towns and villages. Pregnant women were divided into 4 groups according to the presence or absence of Diabetes (Diab) resp. Hypothyroidism (Thyr): Group 0 – 62.7% (n-343) – without Thyr or Diab; Group 1 – 22.9% (n-125) – Thyr; Group 2 – 11% (n-60) – Diab; Group 3 – 3.5% (n-19) – with Thyr and Diab. *Methods*: Fasting morning venous blood (TSH, FT4 - determined by ECLIA method) and fresh morning urine sample (to determine urine iodine concentration - UIC) was taken. A two-hour, 75 g oral glucose tolerance test (OGTT) was performed. The peripheral levels of 25(OH)D were tested using a standard assay in a central laboratory on the day of the sampling. The statistical analysis was conducted using standard SPSS 13.0 for Windows. *Results*: Group 2 were the oldest and Group 1 - the youngest, $P<0.001$. Group 2 as well as Group 3 had significantly higher BMI compared to Group 0 and Group 1, $P<0.0001$ / $P<0.016$. In Group 1 thyroid pathology had manifested itself earlier in the course of pregnancy, while in Group 2 dysglycemia occurred later, $P<0.029$ as well as for group 3, $P<0.004$. There was a significant negative correlation between 25(OH)D with level of fasting plasma glycemia - $P<0.004$, and at 120 minute of OGTT, $P<0.003$. In the group of pregnant women with Hyperglycaemia (n-79), deficiency of UIC (<150 µg / L) was reported in 45.6%, and twice more frequently artificial excess in the UIC level (>500 µg / L), compared with the Group with Normoglycemia (n-368). *Conclusion*: All international guidelines specifically emphasize the main risk factors when pregnant women should be screened for early detection of major endocrine diseases. However, the role of some additional factors, such as deficiency of 25(OH)D and iodine, should not be underestimated.

Keywords: Pregnancy, Hypothyroidism, Diabetes, Age, BMI, Deficiency of 25(OH)D, Deficiency of UIC

1. Introduction

Type 2 diabetes mellitus (T2D) and Hypothyroidism are two major socially significant diseases that affect a large percentage of the world's population [1, 2]. Both diseases develop due to some genetic and external environmental factors, affecting metabolism [3, 4]. Pregnancy causes important metabolic and hormonal modifications. Gestational diabetes (GDM) occurs in about 13% of the pregnant women.

The increased necessity of thyroid hormones leads to Hypothyroidism in 2 - 2.5% of the pregnant women according to Lazarus JH (2014). Various studies have demonstrated that when using a fixed upper reference limit of TSH, about 8-28% of pregnant women show high levels of this hormone [5-7].

Hypothyroidism is one of the most common endocrine diseases during pregnancy, mainly associated with dietary iodine deficiency, especially in countries with low gross domestic product. With the onset of gestation, the level of thyroid hormones is modulated, which may affect the development of pancreatic beta-cells [3]. Several studies have examined the role of thyroid hormones in the development and maturation of pancreatic beta-cells [8]. Thus, the studies of Kemkem Y. et al. (2020) found out that Gestational hypothyroidism affects beta-cell maturation and function, which can affect glucose metabolism. Long lasting hypothyroidism can cause long-term damage to endocrine pancreatic function and development of T2D in the affected individual [9, 10]. After a two-year follow-up of a group of T2D patients, P. Ramulu, et al. (2016) found that 13% developed Subclinical Hypothyroidism (SCHT) during this period and none had Subclinical Hyperthyroidism. This process develops mainly in women [11].

The aim of the present study is to investigate the relationship between Hypothyroidism and Hyperglycemia in Bulgarian pregnant women and to look for the influence of various factors on the manifestation of each of these diseases separately, as well as their role in cases of simultaneous presence of both diseases - Hypothyroidism and Hyperglycemia.

2. Study Design

The study was conducted as a cross-sectional multicenter population-based and was realized from September 25 to November 6, 2019 in 10 regions of Bulgaria (Sofia and Sofia region - Samokov, Pirdop; Smolyan; Gotse Delchev; Gabrovo; Troyan-Apriltsi, Burgas, Stara Zagora, Pleven), including small towns and villages from each region or a total of 84 settlements. Regions with a known iodine deficiency in the past were included, such as Sofia-city, Sofia district, Smolyan, Gotse Delchev, Gabrovo, Troyan, as well as regions with iodine sufficiency in the past - Burgas, Stara Zagora, Pleven and their districts. The study was conducted with the assistance of 104 endocrinologists and gynecologists from selected areas. From their lists of registered pregnant women, 630 were invited to participate, and 547 (86.8%) responded.

3. Material

We studied 547 pregnant women, mean age 30±5 years, median - 30 (18-47), divided into age groups - 40% (28 - 32 years), 23.4% (33 - 37 years), 21% (23 - 27 years), 8.4% (38 - 42 years, 6% (18 - 22 years) and 1.1% (43 - 47 years). The examined pregnant women were also distributed by trimesters resp. gestational week (g.w.) as follows: first - 110 (20.1%) – up to 12 g.w, second - 276 (50.5%) – 13-24 g.w, third - 161 (29.4%) – after 24 g.w. The pregnant women were divided into three groups according to criteria for deficiency, insufficiency and sufficiency in the level of 25(OH)D, accepted by our country and the international organisations. In a previous population-based study, using adaptive regression analysis of the effect of 25(OH)D on PTH levels, we defined a cut-off value for hypovitaminosis D of 20 ng / mL (50 nmol / L) for the whole population [12]. This project had to determine both vitamin D deficiency among Bulgarian population and its connection with gender and age. Levels below 10 ng / mL (25 nmol / L) were referred to as severe deficit, 10 - 20 ng/mL (25 - 50 nmol / L) – a deficit similar to the criteria adopted at the Conference on this topic in Eibsee, Germany in 2012 [13]. In our current study we accepted sufficiency level above 30 ng/mL (75 nmol / L) and introduced additional degree of vitamin D insufficiency – between 20 and 30 ng/mL (50-75 nmol / L), based on the IOM recommendations and Endocrine Society clinical practice guideline [14, 15]. Whereas, the IOM recommendations define sufficiency as vitamin levels above 50 nmol / L (20 ng / mL), the second guidelines suggest levels above 75 nmol / L (30 ng / mL), and both recommendations are in agreement with other expert opinions in the field [16, 17], that the optimum levels of vitamin D should be 75 – 125 nmol / L (≥ 30 ng / mL) in order to preserve bone health. The latter is crucial during pregnancy for the formation of the fetal skeleton. Therefore, in pregnant women we suggest the following categories of 25(OH)D levels: Deficiency:<20 ng / mL, Insufficiency: 20 - 30 ng / mL, Sufficiency:>30 ng / mL.

All participants signed informed consent, approved by the local Ethics Commission at Sofiamed University Hospital, Sofia University "Saint Kliment Ohridski", in conformity with the ethical standards of the Helsinki-1964 Declaration and its later additions [18]. Each pregnant woman filled in a Questionnaire with the help of a specially designated medical person from the "face to face" team in order to collect data on pregnancy history, intake of combined vitamins and minerals, vitamin D, other medications by type and doses, available thyroid or other diseases. Pregnant women were admitted to the Screening at random without pre-selection. 458/547 (83.7%) of them took medicines, mainly magnesium, folic acid and iron (58%), as well as some other drugs given to individual pregnant women in order to preserve the pregnancy and bring it to a successful outcome - spasmolytics, progestins, anticoagulants and antiagregants. Seventy-seven (14.1%) pregnant women took Levothyroxine or received thyrostatic treatment for established thyroid disease. All participants were Caucasians, with no evidence of liver, kidney disease, or malabsorption.

4. Methods

4.1. The Weight and Height, BMI – Before and During Pregnancy; Arterial Pressure

After completing a personal Questionnaire, the current weight and height of each pregnant woman were measured.

The weight before pregnancy was written in the

Questionnaire. The body mass index (BMI – kg / m^2) before pregnancy was calculated, as well as that at the time of screening, i.e. during pregnancy. At addition arterial blood pressure was measured in sitting position after a 5-minute rest.

4.2. Labolatory Tests

4.2.1. TSH, FT4

Laboratory analysis of all blood samples was performed in a Central laboratory on the day of the blood sampling taken in the morning on empty stomach. Serum was quantified on a Cobas e601: TSH analyzer with the ECLIA sandwich method (reference range 0.27 - 4.2 mIU / L); free thyroxine (FT4) with a competitive ECLIA method (reference limits 9.3 - 17.0 ng / L).

4.2.2. OGTT

A two-hour, 75 g oral glucose tolerance test was performed. Venous blood at 0, 60 and 120 minute was taken in plasma tubes, containing Na2EDTA and NaF, as inhibitor of glycolysis for stabilizing glucose in the samples. After centrifugation the samples were transported to the laboratory. All of the samples were analysed in a central laboratory on the day of the blood sampling. Glucose was quantitatively determined using enzymatic reference method with hexokinase (Roche reagent) on Cobas e501 analyzer. The results were in mmol / L.

Established accuracy using human samples and controls:

1) Intra assay: Level 1 (n=6) CV=1.12%; Level 2 (n=6) CV=0.42%
2) Inter assay: Level 1 (n=30) CV=1.25%; Level 2 (n=30) CV=1.58%

Two levels intralaboratory quality control was performed daily.

4.2.3. 25(OH)D

Morning blood sample was taken from the cubital vein and the total levels of 25(OH)D were determined using standard Electro Chemyluminescence Immuno Assay (Competition ECLIA method on Cobas 601 analyzer) in a central laboratory on the day of the sampling. Serum tubes containing separating gel were used. The samples were transported to the lab after centrifugation. The results are determined via a calibration curve in ng / mL. Two levels Intralaboratory quality control was performed daily. The Laboratory participates in two EQA systems – Bulgarian EQAS and INSTAND and has certificates for this parameter.

4.2.4. Urinary Iodine Concentration

Urinary iodine concentration was determined. The pregnant women gave a single portion of the morning fresh midstream urine ~20 ml using clean plastic cups to test iodine. The samples were immediately transported at room temperature in neutral monocuvets to the Central Laboratory (for transport shorter than 8 hours, they were transported at room temperature, which did not influence their quality and the aliquots of all urine samples were frozen at -20° until analysis. The next day the frozen samples were transported in special containers to accredited Limbach laboratory in Heidelberg, Germany. The analysis was performed using the accredited inductively coupled plasma mass spectrometry (ICP-MS) method with the following characteristic: linearity in the range of 0 – 4000 µg / L, precision in the series at 304 mcg / L RSD 0.8%, inter-assay SNU=304 µg / L, 15 shifts, RSD 4.5%; accuracy percentage deviation from adjusted nominal value of the certified reference material SeronormTM Trace Elements urine (SNU) (304 µg / L): 4.0% (data set=4x12), recovery 104%. The results are presented in µg / L.

The Laboratory participates in two EQA systems – Bulgarian EQAS and INSTAND and has certificates for this parameter.

4.3. The Statistical Analysis

Analysis was performed using standard SPSS 13.0 for Windows: descriptive statistics (mean, medians, standard deviation), correlation analysis and analysis of variance (ANOVA, post-hoc test - with Bonferroni alpha correction), using parametrical and non-parametrical methods, including - Chi-Square Test, Fisher's Exact Test, Kolmogorov-Smirnov, Shapiro-Wilk Tests, Levene's Test for Equality of Variances, Student's t-test, Kruskal-Wallis test and Mann-Whitney test. All quantitative variables were presented as mean with standard deviation, median or percentage (unless specified otherwise), p values below 0.05 were accepted as statistically significant.

5. Results

The analysis of the whole group of pregnant women (n-547) showed that the average level of TSH was 2.77±1.84 mIU / L. In the group with Hyperglycemia (n-79) it was 2.56±1.21 mIU / L, and in the group with Normoglycemia (n-468) - 2.80±1.92 mIU / L, NS.

In women with normal thyroid function (n-350), the incidence of Hyperglycemia was 13.1% (46/350). It is noteworthy that in cases of SCHT (n-110) only 4.5% (5/110) of pregnant women have Hyperglycemia. This percentage is about 9 times higher in pregnant women with Clinical Hypothyroidism (CHT) - 41.2% (14/34), who also have Hyperglycemia (NS).

The analysis in the group of pregnant women with Hyperglycemia (n-79) again shows a connection between these two indicators - in 6.3% (5/79) there is also SCHT and in 17.7% (14/79) - and CHT (NS). The distribution of the 547 pregnant women screened in terms of the frequency of two of the main endocrine disorders in this period of a woman's life - Hypothyroidism and Hyperglycemia, is presented in Table 1.

Table 1. *Incidence of cases with Hyperglycemia-alone, Hypothyroidism-alone, as well as both diseases simultaneously or without them for the whole group of 547 pregnant women.*

Disease	number	percentage
Hyperglycemia	79	14.4
Hypothyroidism	144	26.3
Hyperglycemia and Hypothyroidism	19	3.5
Without Hypothyroidism and Without Hyperglycemia	343	55.8
Total	547	100

Thus, it can be summarized that 24% (19/79) of the pregnant women with Hyperglycemia had both Hyperglycemia and

Hypothyroidism, while the remaining 76% (60/79) - had only Hyperglycemia. Moreover, 13.2% (19/144) of the pregnant women with Hypothyroidism had both Hypothyroidism and Hyperglycemia, and the remaining 86.8% (125/144) – had only Hypothyroidism.

On this basis, four groups were formed for detailed analysis - Group 0 (without Hypothyroidism and without Hyperglycemia), Group 1 (Hypothyroidism), Group 2 (Hyperglycemia), Group 3 (with Hypothyroidism and Hyperglycemia), table 2. To simplify the names in the analysis, the abbreviations for Hypothyroidism - Thyr and for Hyperglycemia – Diab, were used.

Table 2. *Distribution of cases with a single disease (Hypothyroidism or Hyperglycemia), with combination of the two diseases or without either of the studied diseases.*

Group Number (%)	Hypothyroidism	Hyperglycemia
Group 0 (without Thyr and Diab), n-343 (62.7)	No	No
Group 1 (Thyr), n-125 (22.9)	Yes	No
Group 2 (Diab), n-60 (11.0)	No	Yes
Group 3 (Thyr and Diab), n-19 (3.5)	Yes	Yes
Total, n-547 (100)		

Table 3. *Age distribution of pregnant women in the four groups.*

Groups	Mean±SD	Median (min-max)
Group 0 (without Thyr and Diab), n-343	30.42±4.88**	30 (18-47)
Group 1 (Thyr), n-125	29.61±5.47*	29 (18-43)
Group 2 (Diab), n-60	32.92±5.37*/**	33 (23-45)
Group 3 (Thyr and Diab), n-19	29.84±4.22	31 (20-36)
Total, n-547	30.00±5.00	30 (18-47)

*P<0.001, **P<0.003

The role of different factors for the frequency of the two diseases separately (Group 1 and Group 2), as well as their manifestation together (Group 3) was studied. The control group was Group 0, in which neither of the two diseases were present.

5.1. Age

Table 3 presents the age distribution in the four groups - mean±SD, median (min-max).

Group 2 (Diab) were the oldest and Group 1 (Thyr) are the youngest (P<0.001),

Group 2 (Diab) were older than Group 0 (without Thyr and Diab), P<0.003.

5.2. Body Mass Index (Before and During Pregnancy)

5.2.1. Body Mass Index Before Pregnancy

After analyzing the Body Mass Index (BMI) before pregnancy according to the mean ranks (Mann-Whitney test) of the different groups, it was found that Group 2 (Diab) had significantly higher BMI compared to Group 0 (without Thyr and Diab) - 268.58 vs 190.35, P<0.0001, and to Group 1 (Thyr) - 119.89 vs 80.09, P<0.0001. This indicator for Group 3 (Thyr and Diab) did not differ from the other three groups, Table 4.

Table 4. *Distribution according to BMI before pregnancy of the four groups of pregnant women.*

Groups	Mean±SD	Median (min-max)
Group 0 (without Thyr and Diab), n-343	22.98±4.58*	21.8 (15.6-50)
Group1 (Thyr), n-125	22.66±4.55**	21.3 (15.2-36.8)
Group 2 (Diab), n-60	26.70±7.09*/**	25.6 (18.2-63.2)
Group 3 (Thyr and Diab), n-19	24.20±4.71	23.8 (18.7-33.5)
Total, n-547	23.36±5.05	22.1 (15.2-63.2)

*P<0.0001, **P<0.0001

5.2.2. BMI During Pregnancy

The analysis of BMI during pregnancy according to the mean ranks (Mann-Whitney test) of the different groups showed that Group 2 (Diab) had a significantly higher BMI compared to Group 0 (without Thyr and Diab) - 279.23 v.s. 188.49, P<0.0001, and to Group 1 (Thyr) - 121.75 v.s. 79.20, P<0.0001. There was also a significant difference between Group 3 (Thyr and Diab) and Group 1 (Thyr) - 93.79 v.s. 69.26, P<0.016, as well as Group 0 (without Thyr and Diab) - 238.34 v.s. 178.35, P<0.016, Table 5.

Table 5. *Distribution of the four groups of pregnant women according to BMI during pregnancy.*

Groups	Mean±SD	Median (min-max)
Group 0 (without Thyr and Diab), n-343	25.43±4.64*/**	24.7 (16.5-49.2)
Group 1 (Thyr), n-125	25.13±4.71*/**	24.4 (17.6-37.2)
Group 2 (Diab), n-60	29.75±6.76*	28.3 (20.4-63.2)

Groups	Mean±SD	Median (min-max)
Group 3 (Thyr and Diab), n-19	27.69±4.14**	27.2 (21.2-36)
Total, n-547	25.91±5.11	25.2 (16.5-63.2)

*P<0.0001, **P<0.016

5.3. Family History of Diabetes and / or Thyroid Disease

5.3.1. Family with Diabetes

In the study group of 547 pregnant women, a family history of diabetes was found in 15.7% (86/547) of them.

Table 6 shows the family history of Diabetes in the four groups. There is more than 5 times higher frequency in Group 2 (Diab) versus Group 1 (Thyr) - 36.7% (22/60) versus 6.4% (8/125)), NS.

Table 6. *Frequency of family burden of Diabetes in the four groups of pregnant women.*

Groups	With Family History (number,%)	Without Family History (number, %)
Group 0 (without Thyr and Diab), n-343	55 (16)	288 (84)
Group 1 (Thyr), n-125	8 (6.4)	117 (93.6)
Group 2 (Diab), n-60	22 (36.7)	38 (63.3)
Group 3 (Thyr and Diab), n-19	1 (5.3)	18 (94.7)
Total, n-547	86 (15.7)	461 (84.3)

The only one case of a family history of diabetes in Group 3 (Thyr and Diab) gives us reason to believe that family history is not a significant factor in these combined cases.

5.3.2. Family with Thyroid Disease

In the study group of 547 pregnant women, a family history of Thyroid disease was found in 20.5% (112/547) of them.

Table 7 presents the distribution of this factor in the four groups of pregnant women. The family burden of Thyroid disease was twice as common as in Group 1 (Thyr) compared to Group 2 (Diab) - 20% (25/125) versus 10% (6/60), NS.

Table 7. *Frequency of family burden of Thyroid disease in all four groups of pregnant women.*

Groups	With Family History (number, (%)	Without Family History (number, (%)
Group 0 (Without Thyr and Diab), n-343	79 (23.1)	264 (76.9)
Group 1 (Thyr), n-125	25 (20.0)	100 (80.0)
Group 2 (Diab), n-60	6 (10.0)	54 (90.0)
Group 3 (Thyr and Diab), n-19	2 (10.5)	17 (89.5)
Total, n-547	112 (20.5)	434 (79.5)

There were only two cases of family history of thyroid disease in Group 3 (Thyr and Diab) - 1.8%, which shows that family history is not a major risk factor when both diseases are present.

5.3.3. Family Burden with Both Diabetes and Thyroid Disease

It turned out that 2.55% (14/547) of the pregnant women had family history of both Thyroid disease and Diabetes. Table 8 shows the distribution of these 14 cases in the four groups.

Within the group of 14 pregnant women with family history of both diseases, the ratios are as follows: one (7.1%) pregnant woman is in Group 1 (Thyr), two (14.3%) pregnant women are in Group 2 (Diab) and the remaining eleven (78.6%) women are in Group 0 (without Thyr and Diab). There is no case of simultaneous family burden in Group 3 (Thyr and Diab).

Table 8. *Frequency of family history of Thyroid Disease and Diabetes for each of the four groups of pregnant women.*

Groups	With Family History (number, (%)	Without Family History (number, (%)
Group 0 (without Thyr and Diab), n-343	11 (3.2)	332 (96.8)
Group 1 (Thyr), n-125	1 (0.8)	124 (99.2)
Group 2 (Diab), n-60	2 (3.3)	58 (96.7)
Group 3 (Thyr and Diab), n-19	0 (0)	19 (100)
Total, n-547	14 (2.6)	533 (97.4)

5.4. Term of Pregnancy - Gestational Week

An assessment was made of the duration of the respective disorder in the four groups according to their mean gestational week (g.w.) - Table 9. In Group 1 (Thyr) thyroid dysfunction appeared significantly earlier in the course of pregnancy, taking into account the mean ranks (Mann-Whitney test) compared to Group 0 (without Thyr and Diab) - 212.78 vs 242.42, P<0.035. The opposite is observed in Group 2 (Diab), in which the metabolic disorder manifested itself significantly later in the course of pregnancy compared to the same Group 0 (without Thyr and Diab) - mean ranks according to (Mann-Whitney test) 232.13 v.s. 196.73, P<0.029. A direct comparison between Group 1 (Thyr) and Group 2 (Diab) again showed earlier onset of thyroid pathology during pregnancy compared to Diabetes - mean rank (Mann-Whitney test) - 84.02 v.s. 111.71, P<0.029.

In Group 3 (Thyr and Diab) hormonal disorders appeared

much later in the course of pregnancy in comparison with Group 1 (Thyr) - mean ranks 97.92 v.s. 68.64, P<0.004, but the metabolic disorders were identical with Group 2 (Diab), i.e. later in the course of pregnancy hormonal and metabolic disorders developed simultaneously - 41.87 v.s. 39.41, NS.

Table 9. *Term for the manifestation of diseases during pregnancy in the four groups of pregnant women (by g.w.).*

Groups	mean±SD (g.w.)	Median (min-max)
Group 0 (without Thyr and Diab), n-343	22.17±8.25*/**	23 (6-38)
Group 1 (Thyr), n-125	20.43±8.15*/**/***	20 (6-40)
Group 2 (Diab), n-60	24.65±7.78**	26 (9-37)
Group 3 (Thyr and Diab), n-19	25.79±7.87***	25 (6-37)
Total, n-547	22.17±8.25	22 (6-40)

*P<0.035; **P<0.029; ***P<0.004

5.5. Reproductive Problems and Adverse Outcome of Pregnancy

Reproductive problems were found in 21.7% (119/547) of the pregnant women, which were distributed as follows: 20.2% (24/119) of the pregnant women from Group 1 (Thyr), 16.8% (20/119) - from Group 2 (Diab) and 2.5% (3/119) of the women from Group 3 (Thyr and Diab), NS. If we look at the frequency within the groups themselves, similar results will be found - Group 1 (Thyr) 19.2% (24/125), Group 2 (Diab) 33.3% (20/60) and Group 3 (Thyr and Diab) 15.8% 3/19) - NS, with the highest incidence of this risk factor for Diabetes.

An unfavorable outcome of a previous pregnancy was reported by 23.7% (130/547) of the pregnant women, distributed as follows: 20.8% (27/130) of the pregnant women from Group 1 (Thyr), 14.6% (19/130) - from Group 2 (Diab) and 3.1% (4/130) from Group 3 (Thyr and Diab) - NS. If we consider the frequency within the groups, a similar ratio will be established - Group 1 (Thyr) 21.6% (27/127), Group 2 (Diab) 31.7% (19/60) and Group 3 (Thyr and Diab) 21% (4/19). Again, this risk factor appears to have the highest incidence in Diabetes.

5.6. High Arterial Blood Pressure During Pregnancy

High arterial bood pressure (AH) was found in 2.4% (13/547) of the pregnant women during their current pregnancy. It turned out that these were 38.5% (5/13) of the women in Group 2 (Diab) and 61.5% (8/13) of the women in Group 0 (without Thyr and Diab). If these two groups are analyzed, the incidence of AH during pregnancy in Group 2 (Diab) will be established in 8.3% (5/60) against 2.3% (8/343) in Group 0 (without Thyr and Diab), NS. Despite the lack of significance due to the very small number of cases, apparently the incidence of AH in Diabetes is higher than in the absence of both diseases (Group 0). Even more important is the correlation found between AH during pregnancy and Hyperglycemia, P<0.013.

5.7. Vitamin D

The whole group of pregnant women studied (n-547) had a mean value of 25(OH)D 25.86±9.46 ng / mL; median 24.51 (7.96-70.00), i.e. there was insuficiency in all four subgroups, Table 10.

Table 10. *Mean level and median of vitamin D - in the whole group and in the four subgroups of pregnant women.*

Groups	Mean±SD	Median (min-max)
Group 0 (without Thyr and Diab), n-343	26.64±9.82*	25 (7.9-70)
Group 1 (Thyr), n-125	23.83±8.62*	22.5 (9.3-47.6)
Group 2 (Diab), n-60	26.33±9.11	24.24 (12.8-60)
Group 3 (Thyr and Diab), n-19	23.64±7.22	21.14 (14-47)
Total, n-547	25.86±9.45	24.51 (8-70)

*P<0.006

Significant difference was found - 245.1 v.s. 205.4, P<0.006. after comparing the mean ranks of Group 0 (without Thyr and Diab) and Group 1 (Thyr). The value of 25(OH)D was even lower in Group 3 (Thyr and Diab), but the small number of cases in it does not allow to establish significant difference with Group 0 (without Thyr and Diab).

According to the presented criteria for determining Sufficiency, Insufficiency and Deficiency of 25(OH)D, the frequency was determined for each of these categories in the whole group of 547 pregnant women. It was found that deficiency was present in 150 (27.4%) pregnant women, Insufficiency - in 252 (46.1%) pregnant women and sufficiency only in 145 (26.5%) pregnant women. The analysis showed that a level of 25(OH)D>30 ng / mL i.e. Sufficiency is twice as rare as in Group 3 (Thyr and Diab) - only in 10.5% (2/19) of the pregnant women in it against 24% (30/125) in Group 1 (Thyr), 23.3% (14/60) in Group 2 (Diab) and 28.9% (99/343) in Group 0 (without Thyr and Diab). The differences are not significant due to the very small number of pregnant women in Group 3 (Thyr and Diab). The opposite is true when assessing the frequency of deficiency + insufficiency of 25(OH)D in the 4 subgroups, namely: the highest frequency is in Group 3 (Thyr and Diab) - 89.5% (17/19) of pregnant women in it against 76.8% (46/60) in

Group 2 (Diab) resp. 76% (95/125) in Group 1 (Thyr) and 71.1% (244/343) in Group 0 (without Thyr and Diab).

5.8. Urine Iodine Concentration

First of all, we must point out that pregnant women in Bulgaria have an optimal level of median urinary iodine concentration (mUIC) of 170 µg / L (95% CI: 161.00 - 177.00) according to our previous study of the same population in 2019 [19]. The studied 547 pregnant women were divided according to the generally accepted criteria for UIC level (deficiency, optimal, over-optimal) into 3 groups, which are presented in Table 11.

Table 11. *Pregnant women with deficiency, optimal and over-optimal level of UIC.*

Level of UIC	Number (Percentage)
Deficiency ≤ 150 µg/L	221 (40.4)
Optimal level 150–249 µg/L	211 (38.6)
Over-optimal level ≥ 250 µg/L	105 (19.2)
Artificial excess>500 µg/L	10 (1.8)
Total	547 (100)

547 pregnant women from the two groups with Hyperglycemia and Normoglycemia were distributed according to UIC level. This is presented in Table 12.

Table 12. *Frequency of Deficiency, Optimal and Over-optimal level according to UIC in pregnant women with Hyperglycemia and Normoglycemia.*

Level of UIC	Hyper-glycemia (Number, %)	Normo-glycemia (Number, %)	Total (Number, %)
Deficiency ≤ 150 µg/L	36 (45.6%)	185 (39.5)	221 (40.4)
Optimal 150-249 µg/L	24 (30.4%)	187 (40.0)	211 (38.6)
Over-Optimal 250-499 µg/L	16 (20.3)	89 (19.0)	105 (19.2)
Artificial excess ≥ 500 µg/L	3 (3.8%)	7 (1.5%)	10 (1.8)
Total	79 (100)	468 (100)	547

According to the data from the Questionnaire 51.2% (280/547) of the pregnant women took combined vitamins with minerals (including iodine) while the remaining 48.8% (267/547) – did not. Only half of the women in the group with Hyperglycemia, took combined vitamins with minerals. Therefore, the intake of vitamins and minerals is not connected with the development of dysglycemia, as only half of the women in the group with Normoglycemia also took vitamins and the rest of them - did not.

6. Discussion

It should be noted that when analyzing the prevalence of diabetes and thyroid dysfunction of the studied population, age, weight, family burden, concomitant diseases and some social factors played an important role [1, 2]. In our study we were very impressed by the fact that while the incidence of Hyperglycemia in the group of pregnant women with SCHT was 4.5%, in the group with CHT this frequency increased 9 times and became 41.2%. In spite of this, due to the small number of cases in the subgroups, this fact underscored the relationship between the two diseases. Meanwhile, Forhead AJ. et al. (2014) concluded that during pregnancy, maternal Hypothyroidism affects glucose metabolism, as well as slowing down fetal growth, impairing maturation of the cardiovascular and nervous systems, and the bones [20].

Interesting are the data from the analysis of The Fremantle Diabetes Study Phase II by Kirsten E. Peters et al (2020), a four-year study of diabetics (T2D-87%, T1D-8% and latent autoimmune LADA-5%). It turned out that 3.4% of the women developed SCHT, 0.2% - CHT and 0.5% - Subclinical hyperthyroidism. There were no significant differences between the types of diabetes. These data support the need for periodic screening for thyroid dysfunction in diabetics [21]. In our material, 24% (19/79) of the pregnant women with Hyperglycemia had both Hyperglycemia and Hypothyroidism, while 13.2% (19/144) of those with Hypothyroidism had Hypothyroidism and Hyperglycemia. It was these 19 pregnant women that we put in a separate Group 3 (Thyr and Diab), trying to determine the factors that led to this situation of double morbidity.

One of the main risk factors for both Hypothyroidism and Diabetes is age. It is a well-known fact that the incidence of both Diabetes and Hypothyroidism increases with age [22, 23].

It was demonstrated that the women in Group 2 (Diab) were significantly older than in Group 1 (Thyr) and Group 0 (without Thyr and Diab) - 32.92±5.37 y vs. 29.61±5.47 y, $P<0.0001$ resp. 32.92±5.37 y vs. 30.42±4.88 y, $P<0.003$. At the same time, the age of the pregnant women in Group 3 (Thyr and Diab) did not differ significantly from that of the other groups.

The weight of the pregnant women, assessed by BMI, is also a significant risk factor for both diseases. We proved this with the present study. An analysis of BMI before pregnancy showed that Group 2 (Diab) had a significantly higher BMI compared to Group 0 (without Thyr and Diab), $P<0.0001$, and Group 1 (Thyr), $P<0.0001$. As for the pregnant women in Group 3 (Thyr and Diab) no significant difference was demonstrated.

It turned out that BMI during pregnancy is actually the most powerful risk factor. Pregnant women from Group 2 (Diab) again had the highest BMI versus Group 1 (Thyr) - $P<0.0001$ and Group 0 (without Thyr and Diab) - $P<0.0001$. However, Group 3 (Thyr and Diab), judging by the mean ranks (Mann-Whitney test), turned out to have a significantly higher BMI compared to the same two groups - 93.79 v.s. 69.26, $P<0.016$ Group 1 (Thyr) resp. 238.34 v.s. 178.35, $P<0.016$ Group 0 (without Thyr and Diab). At the same time, Group 3 (Thyr and Diab) and Group 2 (Diab) did not show a significant difference in terms of this indicator, which led us to the conclusion that high BMI during pregnancy is more closely

related to Diabetes.

The family burden plays imprtan role in both Diabetes and Thyroid diseases. However, after examining the role of this factor in detail, we did not find that it played a significant role among the pregnant population we studied. Family history of diabetes in the group of 547 pregnant women was present in 15.7% (86/547) of them, and for thyroid disease - in 20.5%.

It should be pointed out that the incidence of family history of diabetes in Group 2 (Diab) versus Group 1 (Thyr) is much higher - 36.7% vs. 6.4% (NS). The same ratio is observed with regard to the family burden of thyroid diseases - the frequency is higher in Group 1 (Thyr) against Group 2 (Diab) - 20% vs. 10% (NS). We paid special attention to the family history in Group 3 (Thyr and Diab). It turned out that only 2.55% (14/547) of the pregnant women had a double family burden. The big surprise for us was that only 3 of them had only one disease - one pregnant woman had only Diabetes and two - only Hypothyroidism. The other 11 pregnant women had neither Diabetes nor Hypothyroidism, despite their family history of both diseases. None of the pregnant women in Group 3 (Thyr and Diab) had family burden with the two diseases, which shows unequivocally that family burden is not a factor for the simultaneous presence of these two diseases.

It turned out that the term of pregnancy played significant role in determining the time for the expected manifestation of these diseases during pregnancy. Thyroid disease in Group 1 (Thyr) appeared significantly earlier in pregnancy, taking into account the mean rank of Mann-Whitney test compared to Group 0 (without Thyr and Diab), $P<0.035$. Conversely, Diabetes in Group 2 (Diab) occurred significantly later in pregnancy compared to Group 0 (without Thyr and Diab), $P<0.029$. A direct comparison between Group 1 (Thyr) and Group 2 (Diab) again showed a significantly earlier onset of thyroid pathology during pregnancy compared to Diabetes, $P<0.029$. Hormonal disorders in pregnant women of Group 3 (Thyr and Diab) appeared significantly later in pregnancy compared to Group 1 (Thyr), $P<0.004$, but metabolic disorders in Group 3 (Thyr and Diab) also appeared later than those in Group 2 (Diab) - 25.79±7.87 vs. 24.65±7.78 g.w. (NS). In fact, the disorders in Group 3 (Thyr and Diab) appeared at the latest during pregnancy - an average of 25 g.w. These facts show the connection between the term of pregnancy (g.w.) and hormonal resp. metabolic disorders that may occur in combination with other factors in this period of a woman's life.

Reproductive problems and adverse outcome of previous pregnancies are known risk factors that usually accompany hormonal and metabolic disorders during pregnancy. The frequency of reproductive problems in our material was 21.7% (119/547), while unfavorable outcome of previous pregnancies had 23.7% (130/547) of the studied pregnant women. No significant difference in the frequency of both indicators was found between the compared groups, but their dominance in Group 2 (Diab) was clear. Reproductive problems were reported by 33.3% of the women in Group 2 (Diab) against 19.2% - in Group 1 (Thyr) and resp. in 15.8% of Group 3 (Thyr and Diab), NS. Unfavorable outcome of previous pregnancy was reported by 31.7% of the women in Group 2 (Diab) against 21.6% resp. 21% - from Group 1 (Thyr) resp. from Group 3 (Thyr and Diab), NS. In summary, both problems occurred in over 30% of the pregnant women with Hyperglycemia and in about 20% of those with Hypothyroidism.

The role of arterial hypertension (AH) during pregnancy in relation to these two diseases was also been studied. It turned out that the incidence of AH was 2.4% (13/547) of the studied pregnant women, i.e. it was too low and it would be speculative to look for connections, but still in Group 2 (Diab) there were more pregnant women with AH - 8.3% (5/60). Therefore, in the presence of AH, it is required that the glucose tolerance of the pregnant woman should be tested.

A relationship was sought between the level of 25(OH)D and the studied diseases of the four groups of pregnant women. Insufficiency was found in the level of 25(OH)D, both in the whole group of 547 pregnant women and in each of the four studied groups (Table 10). This fact is not a surprise to us, given our population results from 2012 [12]. The level of 25(OH)D in the 547 pregnant women studied in October fully corresponds to the autumn values for the Bulgarian population from the mentioned study in 2012 – 52.75 nmol / L (95%CI: 50.63 - 54.88) or 21.18 ng / mL [24]. However, it should be noted that the 2012 population-based study included both men and women with a wide age range (20-80 years) and participants were specifically instructed not to take additional vitamin D supplements throughout the year of follow-up. Therefore, the comparison of the two populations would not not be correct, although the values are similar. In our previous analysis of the same group of 547 pregnant women regarding the role of vitamin D supplementation during pregnancy, we found that 51% (278/547) of pregnant women took vitamins during pregnancy, as monotherapy or in combination with other medications. Under these conditions, the incidence of vitamin D deficiency (<20 ng / mL) was significantly higher in pregnant women without vitamin D supplementation - 31.98% versus those with supplementation - 21.94%, $P<0.01$ [25]. In the analysis of the 4 groups in the present study, comparing the average ranks (Mann-Whitney test), a significantly higher level of 25(OH)D was found in Group 0 (without Thyr and Diab) compared to Group 1 (Thyr), $P<0.006$ and in significant for Group 3 (Thyr and Diab), which had the lowest value of 25(OH)D. The small number of cases in this group was not enough to prove significance, although the trend was clear.

The additional correlation analysis showed a negative significant correlation between 25(OH)D with the level of fasting plasma glucose - $P<0.004$, as well as with the plasma glycemia level at 120 minutes at OGTT - $P<0.003$. This unequivocally emphasizes the direct link between vitamin D and the secretory properties of beta-cells, which we studied in 2003 [26]. We believe that vitamin D normalizes beta-cell function by counteracting peripheral insulin resistance and thus overcoming basal hyperinsulinemia. In addition, vitamin D is an important factor in supporting the functional capacity

of beta-cells, judging by its effect on plasma glycemia at the 120th minute of the OGTT performed on pregnant women.

After dividing pregnant women into three groups according to the criteria for Sufficiency (>30 ng / mL), Insufficiency (20 - 30 ng / mL) and Deficiency (<20 ng / mL), it was found that about 27% was the incidence of Sufficiency and as well as of Deficiency. The highest percentage was in the group with Insufficiency - 46%. Sufficient level of 25(OH)D (>30 ng / mL) was present in only 10.5% of the pregnant women in Group 3 (Thyr and Diab), 23.3% - in Group 1 (Thyr), and 24% in Group 2 (Diab) and reached 28.9% in Group 0 (without Thyr and Diab). Due to the small number of participants in Group 3 (Thyr and Diab) no significant differences were demonstrated, but it was clear that in Group 0 the incidence of 25(OH)D Sufficiency was three times higher (almost 29%). It should be emphasized that in Bulgarian pregnant women the level of 25(OH)D follows the population level, as does the distribution in the three categories - Sufficiency, Insufficiency and Deficiency of vitamin D in the general population. It could not be different due to the fact that the level of vitamin D is determined mainly by environmental factors, lifestyle and climatic factors, which are the same for the entire population, including pregnant women, as part of it. It is also a fact that it is necessary for pregnant women to test the level of 25(OH)D and keep it within normal limits. This will ensure quality development of the fetal skeleton after the 16th g.w. On the other hand, insulin resistance develops physiologically during pregnancy, and beta-cell function is impaired. That is why vitamin D would be useful for both peripheral insulin sensitivity and beta-cell functional capacity. Therefore, vitamin D testing in pregnant women should be mandatory part of the usual follow-up Protocol.

We also analyzed the role of urinary iodine concentration for developing impaired glucose tolerance (A detailed analysis of the relationship between UIC and thyroid disease, including hypothyroidism, was presented in our other publications on the same material). The analysis of 79 pregnant women with Hyperglycemia in terms of their Iodine Urine Concentration level and their comparison with the group of 468 pregnant women with Normoglycemia showed several features (Table 12):

1) In pregnant women with Normoglycemia, the distribution between iodine deficiency and sufficiency was the same (about 40% each), and 20% - in the group with over-optimal level.
2) In pregnant women with Hyperglycemia, 30% more were in the iodine deficiency group versus the iodine sufficiency group (45.6% v.s. 30.4%) and 24% were in the over-optimal group.
3) A comparison between the two groups (Normoglycemia and Hyperglycemia) found that in the group with Normoglycemia there were about 25% more pregnant women with iodine sufficiency than in the group with Hyperglycaemia (40% vs. 30.4%, NS).
4) Artificial excess in the level of UIC was found in 10 pregnant women and it turned out to be 2 times more common in the group with Hyperglycemia versus the group with Normoglycemia (3/79 - 3.8% vs 7/468 - 1.5%, NS).

These findings that were supported by the results of a very interesting study by Nathalie Silva de Morais et al. (2020), which found that pregnant women with an over-optimal urine iodine concentration (UIC) of 250 - 499 μg / L had a significantly higher risk of Gestational Diabetes (GDM) compared to the group with an optimal urine iodine concentration of 150 - 249 μg / L [RR 2.90 (CI 1.12-7.45), P<0.027]. The authors concluded that UIC ≥ 250 μg / L was a risk factor for GDM (Relative Risk [RR]=2.9, CI=1.1–7.46, P=0.027) [27].

7. Conclusion

All international guidelines specifically emphasize the main risk factors which require that pregnant women should be screened for early detection of major endocrine diseases [28, 29]. The analysis of our material showed that thyroid pathology could appear early in the course of pregnancy, and in the subsequent period - diabetes mellitus. At the latest in the course of pregnancy, cases with the simultaneous presence of both of these diseases are expected. We should expect more frequent development of disorders of glucose tolerance in older pregnant women. While BMI (before and during the current pregnancy) played a significant role in the manifestation of dysglycemia, BMI (during the current pregnancy) led to the occurrence of both dysglycemia and hypothyroidism. Family burden, reproductive problems, the unfavorable outcome of previous pregnancy, arterial hypertension during current pregnancy, are less important factors for the development of dysglycemia or thyroid dysfunction in our material. An important conclusion could be made about the role of two additional factors - vitamin D deficiency and the role of UIC. With regard to vitamin D, its testing should be included in the usual Protocol for the follow-up of pregnant women, to guarantee the bone health of the mother and fetus. However, optimal vitamin D levels play beneficial role regarding beta-cell function and physiological peripheral insulin resistance during pregnancy, which would reduce the risk of developing glucose tolerance disorders. There is significant inverse correlation between 25(OH)D, the level of fasting plasma glycemia and the glycemia at 120 minutes of OGTT. Therefore, the 25(OH)D level is an important factor for the normal function of beta-cells. Regarding the other factor - UIC, there is higher frequency of iodine deficiency in pregnant women with Hyperglycemia (45.6%), ie. nearly half of this group of pregnant women. On the other hand, it should be noted that the reverse state of artificial excess at the UIC level is twice as common as in the same group of pregnant women with Hyperglycemia. These facts show that maintaining an optimal level of UIC in the entire population, which is a state policy in many countries, will allow not only to maintain a normal level of thyroid hormones before and during pregnancy, but will obviously play a favorable role in terms of glucose tolerance.

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