URINARY IODINE CONCENTRATION: PREDICTOR OF BIRTH WEIGHT OR BIOMARKER FOR ASSESSING THE IODINE STATUS IN HEALTHY PREGNANT WOMEN, ONLY?

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Introduction: This study determined urine iodine concentration (UIC) during gestation, assessed the maternal iodine nutrition status and correlated it with gestational age at birth (GAB) and birth weight (BW).The measurement of UIC provides the best single measurement of the iodine nutritional status in population. Objective: Determination of UIC in pregnant women in North Macedonia. ***Methods:*** This prospective study assessed the iodine nutrition status during the course of pregnancy with reference of median UIC among 364 healthy pregnant women in different gestational age (in trimester and 5-week intervals). ***Results:*** The overall and the 1st to the 3rd trimester median UIC were: 183.7, 207, 189.75 and 169.28 [μg/L], respectively. The median UIC (μg/L) results according to 5-week interval in advancing gestation were: 232.34, 200.13, 152.81, 194.39, 181.28, 160.28, 169.41 and 175.24, respectively. We detected 5.22% (19/364) and 74.72% (272/364) with the median UIC < 50 μg/L and UIC ≥ 100 μg/L, respectively. In multiple regression, the median UIC (β = 0.0000767, P = 0.929) had no statistically significant prediction to the GAB. Disease prevalence results for mean UIC in detecting BW had no statistical significance: area under curve (AUC) = 0.521, z-statistic (0.340), sensitivity (45.83%), specificity (66.27%), predictive (6.59%) and P value (0.734). ***Conclusion:*** Iodine status of pregnant women in our study is generally sufficient by World Health Organization recommendations. The median UIC in each trimester and 5-week interval has statistically insignificant decrease in accordance to the advancing gestation. The median UIC has no significance in predicting GAB and BW.

Key words: pregnancy, urinary iodine concentration, iodine nutritional status, birth weight, gestational age at birth, thyroid metabolism

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INTRODUCTION

Impaired maternal thyroid metabolism and thyroid hormones status are associated with poor outcomes for the mother and the developing newborn, preterm delivery, low birth weight, irreversible damage to the nervous system and intelligence of the fetus (1). Iodine is required for the production of thyroid hormones, which play a crucial role in fetal organogenesis, and in particular in brain development (2). Pregnancy is

associated with substantial changes in thyroid physiology and represents a major stress on maternal homeostasis. The need for iodine in pregnancy is increased (3) due to an increase in maternal thyroxine production to maintain maternal euthyroidism and for transfer of thyroid hormones to the fetus in early pregnancy, before the fetal thyroid begins functioning (4).

The majority of iodine absorbed by the body is excreted in urine. Urine iodine excretion is largely a passive process (5) dependent on glomerular filtration rate (GFR).

The maternal GFR is increased during pregnancy resulting in increased renal loss of ingested iodine, which results with an additional increase in urinary iodine concentration (UIC). In pregnancy, oncotic pressure is substantially decreased because of expansion of the plasma volume, thus contributing to a rise in GFR (6, 7).UIC in nonpregnant women on a stable diet represents a dynamic equilibrium between dietary intakes, thyroidal iodine extraction, the total body thyroid hormone pool, and GFR (5). Pregnancy is a vasodilated state mediated by elevated levels of progesterone.GFR increases continuously within the first month of pregnancy, and reaches its maximum of 40-50% above the level before conception. In the second trimester GFR reached a plateau, and slowly decreased in the third trimester toward the pregnancy concentration (7). Pregnancy is a vasodilated state mediated by alterations in sensitivity to angiotensin II and elevated levels of progesterone. Progesterone has a diuretic effect which is related to aldosterone antagonism which results in increases of GFR (5 - 7). Increased nitric oxide production that occur during normal pregnancy results in cardiac output rising and abets the expansions of plasma volume by stimulating renal sodium and water retention. Both increased renal blood flow and decreased oncotic pressure due to plasma volume expansion contribute to higher GFR (3).

A higher GFR during pregnancy results in decreased circulating creatinine and a possible trend toward lower urinary creatinine concentrations (7, 8). Hence, pregnancy can be expected to result in increased renal iodine losses. In circumstances of borderline or overt iodine deficiency, increases in GFR could deplete total body iodine reserves without the capacity for replenishment if dietary intake remains low (5, 9).The main reasons for increased iodine requirements during pregnancy are: increased thyroid hormone production in pregnancy; the increase in maternal GFR because of increased losses of ingested iodine; fetal and placental consumption of maternal iodine and thyroid hormone proportion. Therefore, the fetal iodine store-supported exclusively by maternal intake, must be continuously refreshed (6).

The excretion of iodine in the urine is a good measure of iodine intake. The median UIC is easily obtainable indicator for iodine status, and it is considered a sensitive marker of current iodine intake that reflects recent changes in iodine status (8, 10).

The measurement of urine iodine excretion provides the best single measurement of the iodine nutritional status of a population (10), but this indicator does not provide direct information about thyroid function (11). UICs are, therefore, not useful for the diagnosis and treatment of individuals, because an individual's UIC can vary daily, or even within the same day but it provides a useful measure of the iodine status of populations (12). UIC can be used as a tool to evaluate the status of iodine nutrition of population (13) and serves as a sensitive parameter of recent iodine intake which reflects the equilibrium between intake and excretion (14). Although there are several methods for UIC quantification reviewed by Dunn *et al.* (15). World Health Organization (WHO) currently recommends the Sandell-Kolthoff-method for epidemiological studies (16). The status of iodine nutrition of a population is determined by measurements of UIC since it is considered an indicator of the adequacy of the iodine intake of that population (5,8,10).

A joint task force of the WHO, the United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) (17) recommends as parameter for the adequacy of the iodine intake in pregnant women, UIC range from 150 to 249 μg/L. UIC less than 150 μg/L have been defined as iodine deficiency (18).

North Macedonia is historically iodine deficient, but due to the long standing and effective preventive measures, it has been considered iodine replete since 2003 (19). Several studies were conducted in 2002, 2003, and 2007 to monitor the iodine status of the Macedonian population and the pregnant women too. These studies have confirmed sustainable sufficient iodine nutrition in the country (20,21).

The aims of this study were divided into primary and secondary. The primary aims were: First, to assess the impact of advancing gestation on UIC in normal pregnancy according to the different determined reference intervals (trimesters or 5 weeks intervals); second, to compare the results of UIC variations over the course of pregnancy with other studies and third, to assess the maternal status of iodine nutrition determined by measurement of UIC and compare it with maternal iodine status in other studies. The secondary aim of our study was to estimate the impact of UIC on some neonatal outcomes [gestational age at birth (GAB) and birth weight].

PARTICIPANTS AND METHODS

Participants

We prospectively investigated UIC in 364 healthy pregnant women in different gestational week (g.w.), without known thyroid disorder that gave birth at the University Clinic of Gynecology and Obstetrics - Skopje. They had a mean age 29.2 ± 5.6 years, and their mean body mass index (BMI) was 27.14 ± 4.79 kg/m^2. They signed an informed consent, and the Ethics Committee of our institution approved the study.

Inclusion criteria were singleton pregnancy in any gestational age without previous history of thyroid disease of the mother or treatment with thyroid drugs. The exclusion criteria were as follows: mothers who smoke cigarettes, mothers with any chronic disease (diabetes mellitus, hypertension), mothers who has personal history of thyroid disease or a visible (palpable goiter). The subjects who took thyroid-related medicine and who had some other gynecologic condition (uterine fibroids and any fetal anomaly diagnosed with amniocentesis or ultrasound) were excluded, too.The data about maternal age, parity, obstetric history and gestational age at the time of birth were noted from the medical history. Birth weight for all newborns was measured by the midwife attending the birth.

Procedures and criteria

A sample of 2 mL of urine was taken with special pipette from each participant and added in Eppendorf tube. Because of within-day and circadian rhythmicity in UI excretion, we collected the urine sample in the same time (fasting morning urine samples) specified time period between 9 to 10 h P.M. (22). The test tubes were marked with identification number (ID) and frozen at T = -20°C, before being transported. UIC in urine samples was analyzed at the National Institute for Health and Welfare (THL) in Helsinki (ICP) by mass spectrometry (MS) using Agilent 7800 ICP-MS system integrated with Agilent SPS 4 auto sampler, with the Pinell-modified Sandell Kolthoff method (23), described previously.

The threshold criteria for UIC data filtering [(UIC < 50 μg/L, UIC ≥ 100 μg/L) in 5 week (wk) gestation intervals group analysis and (150 μg/L < UIC ≤ 249 μg/L in trimester analysis, also (UIC < 150 μg/L) in predictor's analysis] for adequacy of iodine nutrition during calculations were given by WHO, UNICEF and ICCIDD recommendations (16, 17, 24).To assess the iodine status of a population, the median [not the mean ± SD (standard deviation)] UIC is recommended (25). The median, percentiles and interquartile range (IQR) is the preferred measure of central tendency, rather than mean and SD, are most commonly used to describe the distribution of UIC data (17, 26)

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019). Normally distributed variables were presented as mean and SD. Non-normally distributed variables were presented as median and IQR. Some results were presented as N (number) or % (percent). Appropriates Kruskal – Wallis H test or Mann-Whitney U test were used to found difference between UIC values among gestation trimester groups or among 5 wk gestational age interval (between more than 3 groups, or between two groups), respectively. A t Test for independent samples was used to find the difference between symmetrically distributed data. Kernel density plot was created to visualize the distribution of UIC data over a continuous interval. Bivariate Pearson's correlation test was used to measure the strength and direction of relationships between variables. Summary plot of notched box-and-whisker diagram with trend line were created to show UIC results for each 5 wk gestation age period. Multiple backward regression analysis was used to show predictable values of independent variables (maternal BMI, UIC and age as predictors) on the dependent variable GAB and birth weight. Summarized essential information of UIC in meta-analysis according trimester compared with our study, according to the WHO recommendation, was presented as Forest plot diagram. A disease prevalence diagram was created to show prediction value of UIC in detection of birth weight.

RESULTS

During the fourth-month period, from April to July 2017, UIC was assessed in 364 healthy pregnant women in any gestational week (mean age 29.2 ± 5.6 years).

Maternal and fetal outcomes characteristics

Sample characteristics of 364 pregnant women and some of their fetal outcomes are presented in Table 1. In the first trimester of pregnancy (up to 12 g.w.) a total of 67 (18.41 %) were examined, in the second trimester (12 - 28 g.w.) were examined 100 (27.47 %) and in the third trimester (≥ 28 g.w.) were examined 197

(54.12 %). The mean age of the cohort was 29.2 ± 5.6 years, with their mean BMI of 27.14 ± 4.79 kg/m^2 and the mean time of urine sampling was 29.0 ± 10.1 g.w.

The median UIC values in each trimester did not deviate from the median reference values according to the WHO value range (150 – 249 μg/L): in the first (207 μg/L, 95% Confidence Interval [CI] = 197.06 – 221.60), in the second (189.75 μg/L, 95% CI = 181.97 – 217.0) and in the third trimester (169.28 μg/L, 95% CI = 178.76 – 212.7). The overall median UIC during pregnancy (183.7 μg/L) and 95% CI (166.71 to 203.66) were within the WHO's reference range, too.

Appropriate IQR (equal to the difference between 75th and 25th percentiles) for the trimesters are presented in round brackets. We did not found statistically significant difference between median UIC values among trimesters (P = 0.418, T statistic = 1.7447; Kruskal – Wallis H test) and neither between nor within trimester groups (P = 0.747, P = 0.297 and P = 0.289; Mann-Whitney U test). Some of the newborn data (GAB and birth weight) are shown at the bottom of the table 1, too.

The 5th to 95th percentiles range of UIC values for overall, first, second and third trimester of pregnancy were: 48.024 to 438.023 μg/L, 42.493 to 586.963 μg/L, 54.453 to 459.778 μg/L and 44.362 to 422.890 μg/L, respectively. The 25th to 75th percentiles range results for UIC are showed in Table 1, too.

Table 1.
Demographic, clinical and other characteristics according to gestational trimesters

Variables	Mean ± SD Median (IQR)	95% Confidence Interval 25th - 75th percentiles *	group	P - value	group	P - value	group	P - value	
Age (years)	29.2 ± 5.6	28.7 - 29.8							
BMI (kg/m^2)	27.14 ± 4.79	26.64 - 27.63							
Examination time (g.w.)	29.0 ± 10.1	27.9 - 30.2							
UIC (μg/L)	183.7 (161.21)	110.71 - 271.92 *							
UIC (150 - 249 μg/L), WHO[1]	201.5 (47.1)	223.9 - 176.8 *							
UIC (1st trimester)	207 (72.29)	170.39 - 242.68 *	1st	0.747			1st		
UIC (2nd trimester)	189.75 (63.2)	154.60 - 217.8 *	2st		2st	0.297		0.289	
UIC (3rd trimester)	169.28 (50.85)	150.89 - 201.74 *			3st		3st		
GAB (g.w.)	38.4 ± 2.5	38.2 to 38.7							
Birth weight (g)	3127.3 ± 563.4	3068.7 to 3185.8							

SD, standard deviation; IQR, interquartile range; g.w., gestaional week; UIC, Urinary Iodine Concentration;
WHO[1], value range according to the World Health Organization; GAB, gestational age at birth; *, percentiles.

Kernel density plot

A density plot visualizes the distribution of data [UIC (μg/L), x_1 – axis] over a continuous interval or time period. Density trace graph presents distribution and the peak of UIC density, which displays where values of UIC are concentrated. The y-axis (y_1, y_2 and y_3) in a density plot is the probability density function for the Kernel density estimation (KDE). The first (blue, 1), the second (red, 2) and the third (green, 3) line are presenting a distribution of a different data according to the stages of pregnancy. The summary diagram of three different KDE curves is shown on Figure 1.

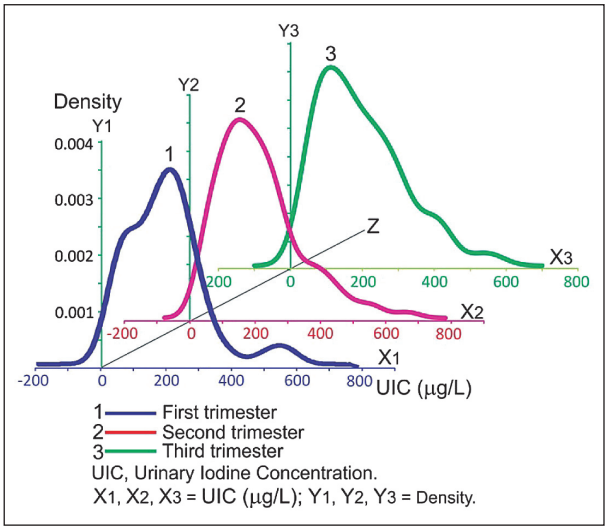

Fig 1. *Kernel density estimation (KDE) and distribution of Urinary Iodine Concentration according to each trimester of pregnancy*

For data density estimation, we used KDE instead histogram, because histogram is not smooth enough to present picture of data distribution as it is KDE. On y-axis is shown the frequency of individuals presented at the corresponding distance – bin. The frequency distribution across space after dispersal event is shown on x-axis: distances from a common origin (binned). The layouts of the frequencies (density from 0 to 0.004) of the UIC have different variability to the value of the mark (UIC). The three KDE curves are positively skewed, or skewed to the right (the mean is greater than the median).

Urinary iodine concentration according to the gestational age

For a more accurate expression of the UIC values variations during pregnancy, we divided the gestation period into 8 subgroups according to a five-week gestational interval. The distribution of UIC with two different UIC threshold values (UIC < 50 μg/L; UIC ≥ 100 μg/L) and Kruskal-Wallis U test between and within groups are shown in Table 2. The UIC results are presented as median according to WHO recommendation (25).

Table 2. *Urinary iodine concentration (UIC) and two different UIC thresholds values in different gestational age groups with results of Kruskal-Wallis H test*

Group	Gestat. Age	N (%)	UIC (μg/L)	UIC < 50 (μg/L)	UIC ≥ 100 (μg/L)	Gestation, wk	Maternal age
Number	wk, [median]	Total 364 (100)	Median (IQR)	N (%), [median]	N (%), [median]	Mean ± SD	Yr, Mean ± SD
1	5 - 9.9 [7]	9 (2.47)	232.34 (149.56)	0	7 (77.7), [258.5]	7.55 ± 1.3	29.1 ± 5.5
2	10 - 14.9 [12]	55 (15,11)	200.13 (142.55)	4 (7.27), [38.41]	39 (70.9), [213.8]	11.49 ± 0.66	29.2 ± 4.9
3	15 - 19.9 [17]	11 (3.02)	152.81 (133.4)	0	7 (63.6), [182.12]	16.8 ± 1.37	31.0 ± 4.7
4	20 - 24.9 [22]	31 (8.51)	194.39 (134.74)	2 (6.45), [42.64]	24 (77.42), [206.98]	21.7 ± 1.26	27.4 ± 6.3
5	25 - 29.9 [28]	61 (16.76)	181.28 (152.15)	2 (3.27), [47.15]	51 (83.61), [214.59]	27.2 ± 1.2	30.6 ± 6.0
6	30 - 34.9 [32]	30 (8.24)	160.28 (167.48)	1 (3.3), [30.6]	21 (70), [230.94]	32.2 ± 1.49	30.5 ± 6.1
7	35 - 39.9 [38]	111 (30.49)	169.41 (177.25)	6 (5.4), [37.5]	86 (77.47), [210.31]	37.7 ± 1.23	28.9 ± 5.4
8	40 - 41.4 [40]	56 (15.38)	175.24 (154.91)	4 (7.14), [40]	37 (66.07), [212.86]	40.41 ± 0.49	28.3 ± 4.8
The results are expressed as: median and interquartile range (IQR), Mean and standard deviation (SD), number N and percent (%). Gest. Age, gestational age; wk, weeks; UIC, Urinary Iodine Concentration;							
Kruskal-Wallis H test							
Test statistic			3.8647				
Corrected for ties Ht			3.8647				
			Degrees of Freedom (DF)		Significance level (P - Value)		
Between Groups			7		0.451		
Within Groups			356		0.795		

The median UIC values in any of the eight gestational age groups did not deviate from the median reference values according to WHO value range (150 – 249 μg/L): 152.81, 160.28, 169.41, 175.24, 181.28, 194.39, 200.13 and 232.34 μg/L, in ascending order, respectively for 3rd, 6th, 7th, 8th, 5th, 4th and 1st gestational age group. There is no statistically significant difference (P = 0.451) in UIC values and maternal age values (P = 0.102) between and within the eight subgroups (Kruskal-Wallis H test and t Test for independent samples, for UIC and age, respectively).

The prevalence of pregnant women in this study with the median UIC < 50 μg/L is only 5.22% (19 cases), and 272 cases (74.72%) from the total were with median UIC ≥ 100 μg/L. Minimal value of median UIC (152.81 μg/L) is registered in third subgroup (15 – 19.9 wk, median 17 wk).

Distribution of the median urinary iodine concentration

The UIC results from each 5-week interval from gestation period [median, 95% CI of the median, 25th percentiles, 75th percentiles and range] are shown by notched box-and-whisker diagram in Fig 2. The median UIC red trend line shows the ascending and descending variation according to the gestational age period. The WHO range determination for UIC (150 – 249 μg/L) is showed by green rectangle. Despite the visible variations of the median UIC during pregnancy showed by red trend line, there is no out of range deviation in UIC, according to the WHO recommendation.

According to the Mann-Whitney test for independent samples we found statistical significance (P = 0.046, test statistic Z = 1.981) between the UIC values in the subgroup A (18 to 21 wk) and the subgroup B (39 to 41.4 wk). The median value for UIC and (95% CI) for the median were 200.85 μg/L (153.62 to 289.85) and 127.27 μg/L (87.17 to 237.99) for subgroup A and B, respectively (Fig. 2).

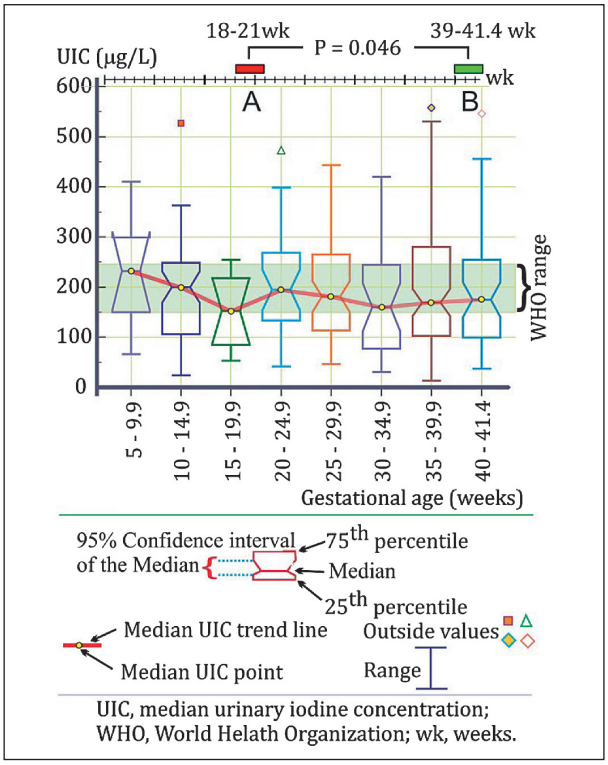

Fig 2. *Distribution of the median urinary iodine concentration through the gestation period*

Bivariate Pearson's correlation analysis

The positive value of Pearson product-moment correlation coefficient (r) as measure of the strength of linear correlation of UIC with maternal and fetal outcome characteristics indicated positive, but not significant correlation between UIC and birth weight (r = 0.05, P = 0.349); UIC and GAB (r = 0.003, P = 0.960); UIC and maternal BMI (r = 0.030, P = 0.568) and UIC and maternal age (r = 0.019, P = 0.72). An inverse significant correlation (P < 0.05) was found between UIC and gestational age of pregnancy (r = - 0.107, P = 0.044).

Multiple backward regression analysis

According to the β standardized Coefficient (βst) and P-value results from multiple backward regression analysis, we found strong positive statistically significant dependency of dependent variable birth weight (βst = 22.5535, P = 0.0004) from maternal BMI as independent variable. This means that any increase of maternal BMI results in an increased birth weight. Independent variables (UIC and maternal age) do not show statistically significant impact on birth weight: (β = 0.1627, P = 0.391) and (β = - 4.7567, P = 0.3782) for UIC and maternal age, respectively.

We found strong inverse statistically significant dependency of dependent variable GAB (β = -0.05560, P = 0.0244) from maternal age as independent variable. This means that any increase of maternal age results in a decreased GAB. BMI (β = 0.004688, P = 0.869) and UIC (β = 0.0000767, P = 0.929) do not show statistically significant predictable value on the dependent variable GAB.

Predictive value of UIC

Selecting option "Plot versus criterion variable (UIC < 150 μg/L)" in MedCalc, we got a curve of disease prevalence i.e. diagram of positive predictive value (%) of UIC < 150 μg/L on birth weight (g). We selected a dichotomous variable (UIC < 150 μg/L) as classification variable: zero (0) for 340 cases with UIC ≥ 150 μg/L and one (1) for 24 cases with UIC < 150 μg/L. Birth weight (g) was selected as estimated variable. The results for positive predictive value variations (%), disease prevalence and associate criterion (birth weight) are shown in Fig. 3.

Disease prevalence was calculated by the next equation: That means.

$$\frac{positive\ cases\ (UIC\ <\ 150mg/\mu gL)}{total\ cases} = \frac{x}{100}\ .\ \text{That means}\ x = \frac{24 \cdot 100}{364} = 6.59\%$$

The maximal sensitivity (45.83%) and specificity (66.27%) of predictor dichotomous variable (UIC < 150 μg/L) in the predicting of birth weight (associate criterion birth weight > 3350 g) is presented as peak (black arrow) of the disease prevalence curve, showed on Fig 3. The receiver operation characteristics (ROC) results were: area under curve (AUC) =0.521, z - statistic = 0.340, P = 0.734, Youden index = 0.121. According ROC, AUC and P – value results, there is no statistical significance in predicting birth weight by classification variable UIC < 150 μg/L.

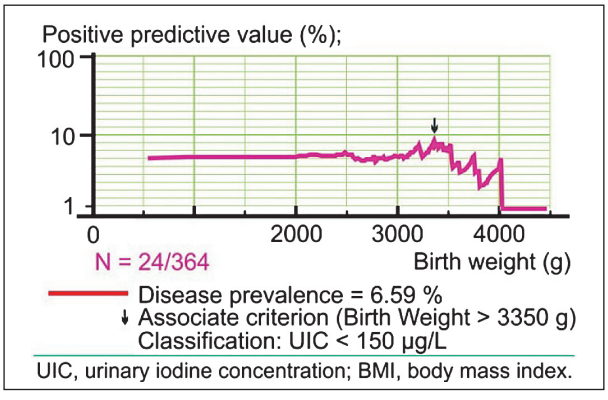

Fig 3. *Prediction value of UIC in detection of birth weight*

Comparison with other studies

The diagram called a forest plot (Fig. 4) summarized essential information of meta-analysis (the name of corresponding author and separate results for median UIC according for each trimester of pregnancy according to the gestation time of urine collection).

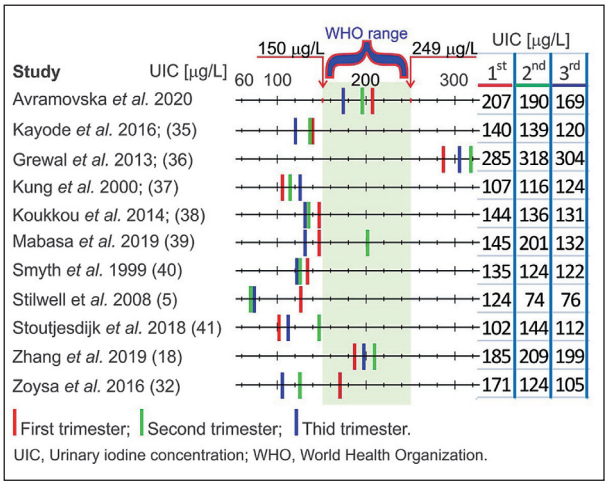

Fig 4. *A forest plot presentation (blobbogram) of median Urinary Iodine Concentration according to the gestation trimester of urine collection*

The vertically placed colored line on numerically divided horizontal line represents the UIC medians for each trimester (red for the 1st, green for the 2nd and blue line for the 3rd trimester). The mutual position of each UIC mean among various studies, as well as their position according to WHO recommended UIC interval (green rectangle, WHO range) for adequate iodine intake in pregnancy, is well understood.

DISCUSSION

We prospectively investigated UIC in 364 healthy pregnant women who consequently came to ambulance of gynecological clinic, regardless of the g.w. of pregnancy, but we selected them by predetermined exclusion criteria. The aims of this study were to determine UIC according to the advancing gestation and to assess the maternal iodine nutrition status, also to correlate the UIC with some neonatal outcomes.

The overall median UIC during pregnancy, median UIC in the first, second and third trimester did not deviate from the median reference value range according to the WHO recommendation (criteria for an acceptable iodine nutritional status in pregnant women) (17). The 25th to 75th percentiles of UIC values in each trimester according to the criteria established by the

WHO indicated an acceptable iodine nutrition status, in women in our study. With the UIC results of 5th to 95th percentiles we detected that 5% of the cohort in our study have median UIC values smaller than 48.0 μg/L (and just as much over 438.0 μg/L). According the results presented in Table 2, only 5.2% from total pregnant women showed UIC < 50 μg/L. Knowing the fact that the adequacy of iodine nutrition is defined by the following criteria: a median UIC ≥ 100 μg/L (with allowed presence 20% of the population having UIC < 50 μg/L) (5, 9), we present adequate population iodine nutrition in our cohort. This percent is almost 4 times smaller than permitted 20% in the general population.

Our results coresponds to the results of Karanfilski *et al.* from 2005-2007, where the median value for UIC for all trimesters in pregnant population was within the interval from 150 - 249 μg/L, which corresponds to an adequate iodine intake (26). These results, compared to the results from their previous study conducted in 2001 (149.7 μg/L for the first, 157.6 μg/L for the second and 130.4 μg/L for the third trimester) suggest an increase in the iodine intake among pregnant women in a population with a confirmed iodine sufficiency (20,26).

However, we must never generalize the given thresholds, range and percent for use in the pregnant population. Changes in iodine requirements and maternal physiology with advancing gestation may invalidate the expected relationship between dietary intake and urine iodine excretion (5, 17). A median UIC of 150 to 249 μg/L has been established to determine the adequate iodine status among pregnant women (17).

Despite the continual downward trend of the mean UIC value from the first to the third trimester, in our study (207, 189.75, 169.28 μg/L), we have not confirmed statistical significant difference neither between nor within the groups (P = 0.418). UIC decreases in the course of pregnancy in our and in most of the previously published studies (27 - 32). During the first trimester and a few weeks later, the fetus relies on maternal thyroid hormones, but as the fetal thyroid gland begins functioning from 15 to 17 weeks gestation, it depends on the maternal iodine supply to maintain thyroid hormone production throughout the remainder of pregnancy (33). The smallest values of the mean UIC (152.81) and IQR (133.4) in the third groups (15 to 19.9 wk, median 17 wk) between series of subgroup's data in our study (Table 2) correlates with requirement of a mother iodine increase confirmed in other studies (5, 30, 33) according to the aforementioned fetal thyroid start-up function. The requirement of a mother iodine increase in pregnancy as result of an increased requirement for thyroxine (T4), a transfer of T4 and iodide from the mother to the fetus and to an increase in the iodine loss due an increase in the renal clearance of iodide (34).

UIC variations during pregnancy sampled by 5 wk intervals are slightly pronounced and gradually downward, so we did not calculate a statistically significant difference neither between (P = 0.451) nor within the groups (P = 0.795). Differences in UIC among gestational groups in studies with inadequate iodine nutrition (depleted iodine status) shown statistical significance (P < 0.001)(5, 33, 37). Unlike them, our and some other studies (18, 32, 39) with better iodine nutritional status, did not showed statistically significant difference between gestational age groups. The most drastic and only one statistically significant (P = 0.046) difference of UIC among two parts of the gestational period A (18 to 21 wk) and B (39 to 41.4 wk) in our study, once again confirms the increased maternal need for iodine during pregnancy.

The trend of median UIC variations throughout pregnancy shown in multiple studies is significantly different: during pregnancy UIC decreases continuously (32, 35, 36, 38, 40); somewhere it increases continuously (37) but elsewhere alternates its trend: first increases from the first to the second trimester, and then decreases from the second to the third trimester (5,18, 36, 39, 41). For better explanation please see a forest presentation shown in Fig. 4. The differences in median UIC values and its trend throughout the pregnancy in the mentioned studies originate from the following characteristics: different time intervals (gestational age) in taking the urine samples (trimesters or 5 wk interval) or in other words diverse referent intervals; difference in the way of taking the urine sample: in what period of the day is it taken (morning, afternoon) and if it was always at the same time, is the sample single (or twice in a day) or is it a collection of 24h urine; differences which are coming from if it UIC results were corrected in accordance with the renal clearance value (GFR); differences that are deriving from the initial UIC value and coming from iodine nutritious status of the pregnant; differences in the number of participants; differences in socio-economy status and ethnic variation, level of education, age and other demographic indicators.

Equalizing the gestational sampling time of 24h urine, UIC correction according to the GFR, assessment of nutritional status with iodine intake and increasing the number of participants are necessary tasks that should be applied so that UIC can be used to assess iodine status in pregnant cohort. The large intra-individual variation in UIC from either spot or 24-hour urine samples means that UIC cannot be used to assess iodine status in an individual pregnant woman. UIC (μg/L) in spot urine samples could to be about

60-65% of the amount excreted in 24 h (42). Thus, multiple factors interact in pregnancy to aggravate of the real UIC value in each examined individual. However, in the absence of clearly defined reference intervals for iodine excretion (UIC) in pregnancy, studies from populations with both adequate iodine nutrition and iodine deficiency provide insight into changes expected in normal pregnancy (5).

We do not found significant correlation between UIC and birth weight (P = 0.349), in accordance with the results of other studies (43, 44). Some studies found positive association between these variables, but these associations were inconsistent across trimesters (45, 46). Therefore, variable, inaccurate with the large intra-individual trimester variation in UIC and non-standardized UIC measurements, make it difficult to correlate with pregnancy outcome. That is why declared inverse correlation between UIC and examination time in our study (P = 0.044) is questionable. In backward multiple regression analysis we found that maternal BMI as independent variable has a positive impact on birth weight (P = 0.0004), only. The included independent variables (UIC and maternal age) do not showed statistically significant impact on birth weight. Including UIC, maternal age and BMI in backward multiple regression analysis for detecting of predictor impact to GAB, we found strong inverse statistically significant dependency of dependent variable GAB (P = 0.0244), only. Opposite to our study results, Rydbeck et al (2014) (47) in cohort of 1617 women [maternal UICs ranged from 0.020 to 10 mg/L (median 0.30 mg/L)], presented that UIC significantly positively associated with birth weight and length for UIC below 1.0 mg/L. Snart *et al.* (2019) (48) collected spot urines samples for UIC in 541 pregnant women with insufficient iodine concentration according WHO. They have not found evidence that UIC is adversely associated with the birth outcomes assessed in their study (48). Due to the different results in our and in the aforementioned studies about UIC association with birth weight, we decided to assess the possible predictive value of UIC on birth weight. We found that there is no statistical significance in predicting birth weight by UIC. Low values of sensitivity and specificity, low AUC (0.521) of predictor dichotomous variable (UIC < 150 μg/L) in predicting birth weight results with no statistical significance (P = 0.734).

More extensive analysis of fetal outcome prediction and analysis of UIC correlation with other iodine and infant parameters was not the main aim of our study, but it may be the motive and goal for future studies on a similar topic.

Our study has several strengths. First, our cohort includes 364 pregnant women, a relatively large sample size for studies of spot urine. Second, we used the Pinell-modified Sandell Kolthoff ICP-MS method, which is a gold standard for quantifying urine iodine. Third, we collected fasting urine spot samples in the same, specified time period (9 to 10h, P.M.) to avoid UIC within-day and circadian rhythmicity variation in UI excretion. Fourth, the UIC results are shown by both, trimester and a 5-week gestational age interval, joined in one study.

STUDY LIMITATIONS

Several limitations to this study should be considered. Analyzing a single spot urine sample instead of multiple spot urinary collections or more efficient repeated 24-hour collections is the first and the main lack in our study. The second limitation is that we did not measure urine creatinine levels to provide UI to creatinine ratio (UI/Cr), as an indicator for assessment of the adequacy UIC, because the serum iodine changes are similar to the UI/Cr. The UIC results in our study are not corrected according to the GFR, which is the third limitation. The fourth and last limitation is the different number of participants in trimester and 5-week gestational groups which further reduces the real estimate of UIC.

CONCLUSIONS

We have demonstrated that the iodine status of pregnant women in our study cohort is generally sufficient by WHO recommendations. The median UIC decreased from the first to the third trimester during pregnancy, but not with statistical significance. The overall median UIC values and median UIC in each trimester did not deviate from the median reference values according to the WHO guidelines, also in any of the eight 5-week gestational age groups.Evident decrease of median UIC is observed in 5-week gestational age group during pregnancy, which is also statistically insignificant.

The most pronounced descending decline in the UIC trend curve registered in the section from 5 to 20-week interval and it's milder decrease to the end of pregnancy is in line with maternal and fetal physiology of iodine needs.

We found strong inversely dependency of GAB from maternal age, but not from UIC and BMI, and strong positive dependency of birth weight from maternal BMI, but not from UIC and maternal age. Because the reference interval for UIC to each trimester or 5-week interval of pregnancy is not established,it is difficult to make an appropriate assessment of correlation of the

UIC and birth outcomes. The median UIC has no significance in predicting birth outcome, but is of great importance for assessing iodine status in pregnant population, more for assessment of population iodine nutrition status, than for individual assessment for it. The validity of a single urine sample for the assessment of iodine status in pregnancy and its impact on birth outcomes warrants further research.

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SAŽETAK

KONCENTRACIJA JODA U MOKRAĆI: PREDSKAZATELJ PORODAJNE TEŽINE ILI BIOLOŠKI BILJEG ZA PROCJENU JODNOG STATUSA SAMO U ZDRAVIH TRUDNICA?

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Uvod: Ova je studija utvrdila koncentraciju joda u mokraći (UIC) tijekom trudnoće, procijenila prehrambeni status joda kod majke i povezala ga s gestacijskom dobi pri rođenju (GAB) i porođajnom težinom (BW). Mjerenje UIC-a omogućava najbolje pojedinačno mjerenje prehrambenog statusa joda u populaciji. ***Cilj:*** Određivanje UIC-a trudnicama u sjevernoj Makedoniji. ***Metode:*** Ova prospektivna studija procjenjivala je prehrambeni status joda tijekom trudnoće, pozivajući se na medijan UIC 364 zdrave trudnice u različitoj gestacijskoj dobi (u intervalima tromjesečja i 5 tjedana). ***Rezultati:*** Ukupna i prosječna UIC od 1. do 3. tromjesečja bila su: 183,7, 207, 189,75 i 169,28 [µg / L]. Srednji rezultati UIC (µg / L) prema intervalu od 5 tjedana u napredovanju trudnoće bili su: 232,34, 200,13, 152,81, 194,39, 181,28, 160,28, 169,41 i 175,24. Otkrili smo 5,22 % (19/364) i 74,72 % (272/364) s medijanom UIC <50 µg / L, odnosno UIC ≥ 100 µg / L. U višestrukoj regresiji, medijan UIC (β = 0,0000767, P = 0,929) nije imao statistički značajno predviđanje za GAB. Rezultati prevalencije bolesti za srednji UIC u otkrivanju BW nisu imali statističku značajnost: područje ispod krivulje (AUC) = 0,521, z-statistika (0,340), osjetljivost (45,83 %), specifičnost (66,27 %), prediktivna (6,59 %) i P vrijednost (0,734). ***Zaključak:*** Jodni status trudnica u našem istraživanju u pravilu je dovoljan prema preporukama Svjetske zdravstvene organizacije. Medijan UIC-a u svakom tromjesečju i intervalu od 5 tjedana statistički je beznačajno smanjen u skladu s napredovanjem trudnoće. Medijan UIC nema značenje u predviđanju GAB i BW.

Ključne riječi: trudnoća, koncentracija joda u mokraći, prehrambeni status joda, težina rođenja, gestacijska dob pri rođenju, metabolizam štitnjače