SUFFICIENT IODINE INTAKE IN SCHOOLCHILDREN FROM THE ZAGREB AREA: ASSESSMENT WITH DRIED BLOD SPOT THYROGLOBULIN AS A NEW FUNCTIONAL BIOMARKER FOR IODINE DEFICIENCY

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SUMMARY – Current methods for assessment of iodine intake in a population comprise measurements of urinary iodine concentration (UIC), thyroid volume by ultrasound (US-Tvol), and newborn TSH. Serum or dried blood spot thyroglobulin (DBS-Tg) is a new promising functional iodine status biomarker in children. In 1996, a new act on universal salt iodination was introduced in Croatia with 25 mg of potassium iodide *per* kg of salt. In 2002, Croatia finally reached iodine sufficiency. However, in 2009, median UIC in 101 schoolchildren from Zagreb, the capital of Croatia, was 288 µg/L, posing to be excessive. The aim of the study was to assess iodine intake in schoolchildren from the Zagreb area and to evaluate the value of DBS-Tg in schoolchildren as a new functional biomarker of iodine deficiency (and iodine excess). The study was part of a large international study in 6- to 12-year-old children supported by UNICEF, the Swiss Federal Institute of Technology (ETH Zurich) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD). According to international study results, the median cut-off Tg <13 µg/L and/or <3% Tg values >40 µg/L indicate iodine sufficiency. The study included 159 schoolchildren (median age 9.1±1.4 years) from Zagreb and a nearby small town of Jastrebarsko with measurements of UIC, US-Tvol, DBS-Tg, T4, TSH and iodine content in salt from households of schoolchildren (KI/kg of salt). Overall median UIC was 205 µg/L (range 1-505 µg/L). Thyroid volumes in schoolchildren measured by US were within the normal range according to reference values. Median DBS-Tg in schoolchildren was 12.1 µg/L with 3% of Tg values >40µg/L. High Tg values were in the UIC range <50 µg/L and >300 µg/L (U-shaped curve of Tg plotted against UIC). All children were euthyroid with geometric mean TSH 0.7±0.3 mU/L and arithmetic mean T4 62±12.5 nmol/L. The mean KI content *per* kg of salt was 24.9±3.1 mg/kg (range 19-36 mg/kg). Study results indicated iodine sufficiency in schoolchildren from the Zagreb area. Thyroglobulin proved to be a sensitive indicator of both iodine deficiency and iodine excess in children. Iodine content in salt from households of schoolchildren was in good compliance with the Croatian act (20-30 mg KI/kg of salt).

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Introduction

Sufficient iodine intake is especially important for the entire population, since iodine is the main constituent of thyroid hormones. Iodine deficiency (ID) has many adverse effects on growth and development, most important being mental impairment in children[1,2]. The most susceptible population groups include pregnant and breastfeeding women and small children because of the role of thyroid hormones in brain development, especially for normal neuronal migration and myelinization of the brain during fetal and early postnatal life. Hypothyroxinemia during these critical periods causes irreversible brain damage, with mental retardation and neurological abnormalities[3]. People living in areas affected by severe ID may have an intelligence quotient (IQ) of up to 13.5 points below IQ values recorded in communities without ID[4]. Salt iodization is the most widely used strategy to control and eliminate iodine deficiency disorders (IDD)[5]. Salt iodization programs require an effective system of monitoring and evaluation. Measurement of iodine content in salt is the main process indicator. Impact indicators to assess iodine status of the population comprise urinary iodine concentration (UIC) measurements, goiter rate, newborn thyrotropin (TSH) and blood thyroglobulin (Tg)[2,5]. However, each iodine status indicator has limitations. Median UI is the main indicator to be used to assess iodine status of a population. Urinary iodine is an indicator of recent iodine intake but not of thyroid function. TSH is a sensitive indicator of iodine status only in the newborn period. Thyroglobulin assay for dried blood spots (DBS-Tg) is being increasingly used for monitoring of iodine status in school-aged children[6], since it was recommended by the WHO/UNICEF/ICCIDD in 2007[5]. In iodine deficient areas, serum Tg is elevated due to TSH hyperstimulation and thyroid hyperplasia[2]. Serum Tg is well correlated with the severity of iodine deficiency as assessed by UI[7]. In intervention studies in children, Tg falls rapidly with iodine repletion and is a more sensitive indicator of iodine repletion than TSH or T4[2,8]. Fall in Tg values has been observed within several months after iodine repletion[9,10]. In a large international project with more than 2000 children from 12 countries included in the survey, from iodine deficient to excessive, DBS-Tg was a sensitive biomarker of optimal thyroid function within the UIC range in schoolchildren from 100 to 299 μg/L (adequate and more than adequate iodine intake according to WHO recommendations)[11]. There was a higher prevalence of elevated Tg values in children with ID (UIC <100 μg/L) and iodine excess (UIC >300 μg/L). Therefore, Tg is a good biomarker of ID and iodine excess. Cut-off median Tg <13 μg/L suggests adequate iodine supply. However, the prevalence of elevated Tg values (above 40 μg/L) should not be more than 3% in children[11]. In the past, Croatia was a region of severe ID with the presence of endemic cretinism. First regulation on universal salt iodization (USI) in Croatia was introduced in 1953 with 10 mg of potassium iodide *per* 1 kg of salt. As a result, a threefold reduction in goiter prevalence with disappearance of cretinism was reported ten years later[12]. However, at the beginning of 1990s, mild to moderate ID still persisted in Croatia[12-14]. In 1996, a new regulation on USI was introduced in Croatia with 25 mg of potassium iodide *per* 1 kg of salt. In 2002, Croatia finally reached iodine sufficiency[15]. Croatian model of salt iodization is USI program that implies general iodization of all human and livestock salt, including salt used in food industry according to the recommendations of the World Health Organization (WHO), the International Council for Control of Iodine Deficiency Disorders and UNICEF[5]. With the successful elimination of ID and a good program of iodine prophylaxis, Croatia has been internationally recognized as a model country for solving this important public health issue. The latest nationwide iodine survey carried out in 2009 demonstrated sufficient iodine intake in Croatia with the overall median UIC in schoolchildren of 248 μg/L and median UIC in pregnant women of 159 μg/L. The median UIC in 101 schoolchildren attending the Otok School in Zagreb was 288 μg/L. Thyroid volumes in school-boys and school-girls from Zagreb were within the normal range according to reference values[16].

The aim of the study was to assess iodine intake in schoolchildren from the Zagreb area, the capital of Croatia, and to evaluate the value of DBS-Tg in schoolchildren as a new functional biomarker of iodine deficiency and iodine excess. The study was part of a large international study in 6- to 12-year-old children supported by UNICEF (New York, USA), the Swiss Federal Institute of Technology (ETH) (Zurich, Switzerland) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) (Zurich, Switzerland)[11].

Subjects and Methods

The study sample included 159 healthy 6- to 12-year-old elementary schoolchildren living in the capital of Zagreb and the neighboring small town of Jastrebarsko. Two schools were selected to participate, Otok School from Zagreb and Ljubo Babić School from Jastrebarsko. The selection of schools was purposeful because of the large number of schoolchildren attending these schools and a previous study from 2009[16], which demonstrated more than adequate iodine intake in schoolchildren from Otok School in Zagreb (median UIC 288 μg/L) based on the WHO criteria for median UIC[5]. The sources of dietary iodine were local foods and variable amounts of iodized salt. A total of 78 boys and 81 girls were included in the survey. Exclusion criteria were age <6 years or >12 years, chronic diseases, and use of chronic medications or iodine supplements. Ethics Committees of the ETH Zurich, Switzerland, and of the Sestre milosrdnice University Hospital Center, Zagreb, Croatia approved the study protocol. Data collection was carried out between 2010 and 2012.

The study design was cross-sectional. At the schools, body height and weight were measured using the standard anthropometric technique[17]. Heights were recorded to the nearest mm and weights to the nearest 100 g. Spot urine samples were obtained from the children, aliquoted and stored at -20 °C until analysis. Whole blood from a finger stick was spotted onto filter paper (grade 903; Schleicher & Schuell, Dassel, Germany) and allowed to dry at room temperature for 24 h. Dried blood samples were stored at 4 °C in sealed low-density polyethylene bags until shipment to Zurich for analysis. Schoolchildren were informed to bring from home 100-150 g of household salt in plastic boxes for analysis of iodine content in salt. A total of 108 salt samples were analyzed for iodine content. Salt producers were marked at each salt sample from households of the study schoolchildren.

Laboratory analysis

Urinary iodine concentration was measured using a modification of the Sandell-Kolthoff reaction[18]. DBS were analyzed for TSH (Delfia NeoTSH)[19] and total thyroxine (T4) (Delfia Neonatal T4 kit), both from PerkinElmer Life Sciences (Turku, Finland). Normal reference values for whole blood samples are as follows: TSH, 0.2-3.7 mU/L, and total T4, 65-165 nmol/Liter. For analysis of DBS-Tg, a two-site dissociation enhanced lanthanide fluorescent immunoassay (Delfia) serum Tg assay (PerkinElmer Life Sciences, Wallac, Turku, Finland), adapted for DBS, was used[6].

Thyroid volumes in schoolchildren were measured by ultrasound. Ultrasonography of the thyroid gland was performed with a real-time linear transducer (Tossbee, Toshiba, Japan) using 7.5-10 MHz. Thyroid volume was calculated with the following equation, according to Brunn and Block[20]: $V=0.479[(a1b1c1) + (a2b2c2)]$, where a represents length, b width, c breadth, 1 right lobe and 2 left lobe. Data were compared with the international reference values for thyroid volume in iodine-sufficient schoolchildren[21]. Iodine content in salt was measured in the Croatian Institute of Public Health by titration method[22].

Statistical analysis

Data processing and statistics were done using the IBM SPSS statistics version 20. Subjects with all data (UIC, thyroid function tests and US) were included in the analysis. Non-normally distributed data were log-transformed for further analysis. For parameters

Table 1. Subject characteristics, urinary iodine concentration (UIC) and dried blood spot thyroglobulin (Tg)

N	Gender (M/F)	Age (yrs)[1]	Height (m)[1]	Weight (kg)[1]	UIC (μg/L)[2]	Tg (μg/L)[3]
159	78/81	9.1±1.44	1.40±0.10	35.5±10.5	205 (1-505)	11.3±10.5

[1]arithmetic mean ± SD; [2]median (min-max), [3]geometric mean ± SD

Table 2. Thyroid function in 6- to 12-year-old children according to WHO categories of urinary iodine concentration (UIC)

UIC (μg/L)	<50 μg/L Moderate to severe iodine deficiency	50-99.9 μg/L Mild iodine deficiency	100-199.9 μg/L Adequate iodine intake	200-299.9 μg/L More-than-adequate iodine intake	>300 μg/L Excess iodine intake
n	15	8	54	57	25
TSH (mU/L)[1]	0.65±0.18	0.73±0.39	0.63±0.32	0.74±0.35	0.58±0.19
T4 (nmol/L)[2]	64.70±16.16	70.0±11.58	63.0±11.18	59.60±11.26	64.3±14.58
Tg (μg/L)[1]	20.51±14.14	12.32±11.81	7.56±4.21	10.13±6.19	23.44±11.72
Tg >40 μg/L (%)	13.3	12.5	0	0	8.0

[1]geometric mean ± SD; [2]arithmetic mean ± SD; TSH = thyrotropin; T4 = thyroxine; Tg = thyroglobulin

including values between 0 and 1 (TSH and Tg), a constant of 1 was added to the values before transformation. Arithmetic mean ± SD was used to report normally distributed data and geometric mean ± SD for data that were normally distributed after log-transformation. Spearman correlations were calculated between UIC and thyroid function markers and the Loess smoothed line calculation (with 60% of points to fit) was used to describe the best fit of the thyroid function markers plotted against UIC. The level of significance was set at $p<0.05$.

Results

General subject characteristics are shown in Table 1. Data on 159 subjects were analyzed. Urine samples for determination of UIC, as well as DBS for the measurement of TSH, T4 and Tg were available from all children. There were 78 boys and 81 girls. Overall median UIC in schoolchildren was 205 μg/L (range 1-505 μg/L). Most of the schoolchildren had UIC in the range of 100-199.9 μg/L and 200-299 μg/L (34.0% and 35.8%, respectively) (Fig. 1) (adequate and more than adequate iodine intake according to the WHO/UNICEF/ICCIDD categories). All children were euthyroid with geometric mean TSH 0.7±0.3 mU/L

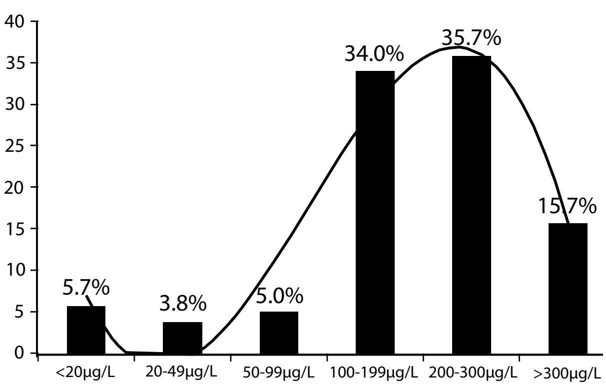

Fig. 1. Distribution of urinary iodine concentration (UIC) in schoolchildren from the Zagreb area. Overall median UIC was 205 μg/L. Second polynomial order trend line was added to show the best fit.

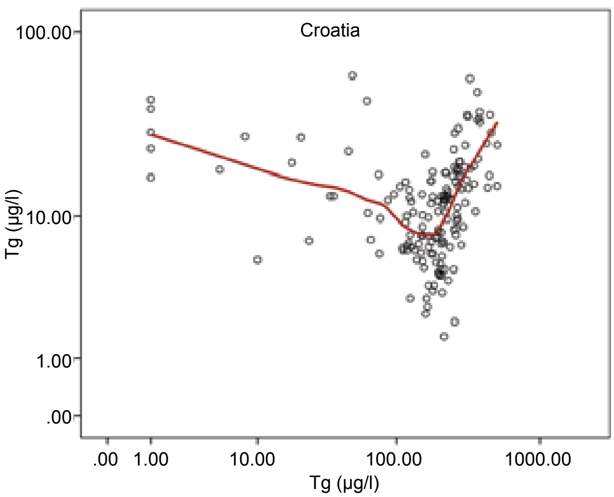

Fig. 2. Scatterplot (using individual values of 159 children aged 6 to 12 years from Zagreb and Jastrebarsko) of dried blood spot thyroglobulin (Tg) vs. urinary iodine concentration (UIC) with the Loess smoothed line added to show the best fit. Data are presented on a log scale for Tg and UIC.

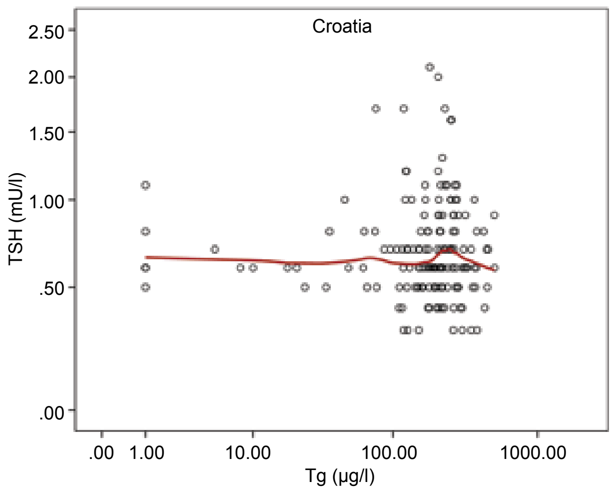

Fig. 3. Scatterplot (using individual values of 159 children aged 6 to 12 years from Zagreb and Jastrebarsko) of thyrotropin (TSH) vs. urinary iodine concentration (UIC) with the Loess smoothed line added to show the best fit. Data are presented on a log scale for UIC.

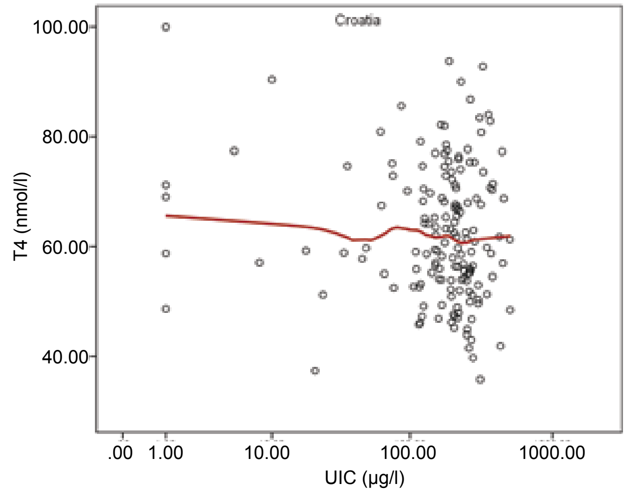

Fig. 4. Scatterplot (using individual values of 159 children aged 6 to 12 years from Zagreb and Jastrebarsko) of total thyroxine (T4) vs. urinary iodine concentration (UIC) with the Loess smoothed line added to show the best fit. Data are presented on a log scale for UIC.

and arithmetic mean T4 62±12.5 nmol/L. Geometric mean Tg concentration was 11.3±10.5 μg/L. Table 2 shows thyroid functions according to the WHO/UNICEF/ICCIDD categories of UIC used to classify iodine intake in a population of school-aged children. Differences in Tg were most pronounced. Using a cut-off median DBS-Tg <13 μg/L published in the international study by Zimmermann *et al.*[11], in which this study also took part, high Tg values were found in UIC range <50 μg/L and UIC range >300 μg/L ($p<0.05$). Median DBS-Tg was 12.1 μg/L with 3% of Tg values >40 μg/L. None of the schoolchildren in the adequate and more than adequate UIC range had Tg >13 μg/L. Figures 2, 3 and 4 show plots of T4 and log TSH and Tg against log UIC including the Loess smoothed line depicting the best fit. Arithmetic mean KI content *per* kg of salt was 24.8±3.1 mg/kg (range 19-36 mg/kg). Thyroid volumes were measured by US in 152 schoolchildren. Thyroid volumes were within the normal range in school-boys and school-girls according to reference values established by Zimmermann *et al.*[21].

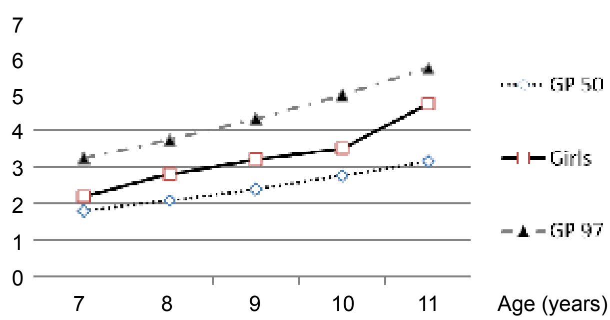

Fig. 5. Thyroid volumes in school-girls from the Zagreb area. Reference median [50th percentile (GP 50)] and 97th percentile (GP 97) values for thyroid volume in girls according to age as established by Zimmermann et al[21].

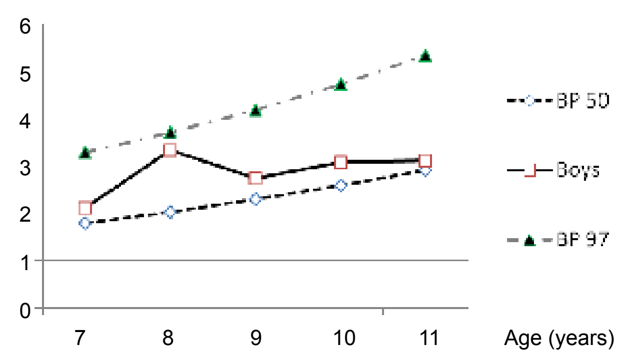

Fig. 6. Thyroid volumes in school-boys from the Zagreb area. Reference median [50th percentile (BP 50)] and 97th percentile (BP 97) values for thyroid volume in boys according to age as established by Zimmermann et al[21].

Discussion

Study results confirmed adequate iodine supply in schoolchildren from the Zagreb area, as assessed by UI, Tvol, as well as with the new functional biomarker DBS-Tg. Median UIC in schoolchildren was 205 μg/L. A total of 69.8% of schoolchildren had UIC in the range of 100-299 μg/L, while 15.7% had UIC >300 μg/L. This study clearly presented the value of DBS-Tg as a good biomarker of iodine deficiency and iodine excess in schoolchildren. In a small subsample of schoolchildren, Tg concentration showed U shaped curve, as previously published in a large international study with participation of subjects from this investigation[11]. Median DBS-Tg of 12.1 μg/L in schoolchildren from the Zagreb area indicated adequate iodine supply (as defined by cut-off median Tg <13 μg/L). The prevalence of elevated Tg values (above 40 μg/L) was 3%. There was a significantly higher prevalence of elevated Tg values in children with ID (UIC <100 μg/L) and iodine excess (UIC >300 μg/L). None of the schoolchildren in the UIC range between 100 and 299 μg/L had elevated DBS-Tg (above 40 μg/L). However, 13.3% of schoolchildren with UIC <50 μg/L, 12.5% of schoolchildren with UIC 50-99 μg/L, as well as 8.0% of schoolchildren with UIC >300 μg/L had Tg >40 μg/L. A sharp ascending part of Tg curve plotted against UIC was observed with rising UIC values, as most of the schoolchildren had UIC in the adequate, more than adequate and excessive range. Study results confirmed DBS-Tg to be a sensitive biomarker of optimal thyroid function within the UIC range of adequate and more than adequate iodine intake according to the WHO recommendations. Therefore, acceptable range of median UIC for children can be widened in the range of 100-299 μg /L for children in revised program monitoring guidelines, as previously suggested[11]. There was no significant change in TSH and T4 at all levels of iodine intake. The problem with serum Tg measurement is the presence of serum thyroglobulin antibodies (TgAb) that may interfere with Tg assays. However, according to the international study results[11], thyroid autoimmunity in schoolchildren was rare at all levels of iodine intake. DBS-Tg complements the use of UIC to measure recent iodine intake and Tvol to assess long-term response. Measurements of iodine content in salt samples from households of schoolchildren demonstrated adequate iodine content in salt in almost all samples (between 20 and 30 mg of KI *per* kg of salt). Assessment of iodine intake in pregnancy is of utmost importance[23]. The value of serum Tg as a biomarker of iodine deficiency in pregnancy has not yet been confirmed. A few studies have been published with different methodology applied. Median Tg <13 μg/L and median UIC ≥150 μg/L were used as cut-off values to define adequate iodine intake in pregnancy[24].

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Sažetak

DOSTATAN UNOS JODA U ŠKOLSKE DJECE S PODRUČJA ZAGREBA: PROCJENA ODREĐIVANJEM TIREOGLOBULINA U SUHOJ KAPI KRVI KAO NOVOG FUNKCIONALNOG BILJEGA NEDOSTATNOG UNOSA JODA

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Metode koje se primjenjuju za procjenu unosa joda u populaciji su mjerenje izlučivanja joda mokraćom (*urinary iodine concentration*, UIC), procjena stanja gušavosti mjerenjem volumena štitnjače ultrazvukom (Tvol) i tireotropina (TSH) u novorođenčadi. Određivanje biljega tireoglobulina (Tg) u serumu ili suhoj kapi krvi je novi obećavajući funkcionalni biljeg stanja unosa joda u djece. Novi zakon o obveznom univerzalnom jodiranju soli uveden je u Hrvatskoj 1996. s 25 mg kalij-jodida po kilogramu soli. Hrvatska je 2002. g. postigla dostatan unos joda. Međutim, 2009. godine medijan izlučivanja joda mokraćom u školske djece iz Zagreba iznosio je 288 µg/L, što je blizu granice prekomjernog unosa joda. Cilj istraživanja bio je odrediti unos joda u školske djece s područja Zagreba i vrijednost određivanja Tg u suhoj kapi krvi u školske djece kao novog funkcionalnog biljega nedostatnog i/ili prekomjernog unosa joda. Istraživanje je dio velikog međunarnodnog projekta UNICEF-a, Švicarskog federalnog instituta tehnologije (ETH Zürich) i Međunarodnog vijeća za kontrolu poremećaja uzrokovanih nedostatkom joda (ICCIDD). Prema rezultatima međunarodnog projekta, granični prijelomni (*cut-off*) medijan Tg <13 µg/L i/ili <3% rezultata Tg >40 µg/L upućuju na dostatan unos joda. U istraživanje je bilo uključeno 159 školske djece (medijan dobi 9,1±1,4 god.) iz Zagreba i obližnjeg gradića Jastrebarsko. Istraživanje je provedeno određivanjem UIC, Tvol, Tg u suhoj kapi krvi, T4, TSH i sadržaja joda u soli iz kućanstava školske djece (KI/kg soli). Ukupni medijan UIC iznosio je 205 µg/L (raspon 1-505 µg/L). Volumeni štitnjače u školske djece izmjereni ultrazvukom bili su unutar granica normale u usporedbi s referentnim vrijednostima. Medijan Tg u suhoj kapi krvi iznosio je u školske djece 12,1 µg/L, a 3% rezultata Tg bilo je >40 µg/L. Visoke vrijednosti Tg zabilježene su u rasponu vrijednosti UIC <50 µg/L i >300 µg/L (U-oblik krivulje Tg u odnosu na UIC). Sva djeca su bila u eutireozi s geometrijskom sredinom TSH 0,7±0,3 mU/L i aritmetičkom sredinom T4 62±12,5 nmol/L. Prosječni sadržaj kalij-jodida po kg soli iznosio je 24,9±3,1 mg/kg (raspon 19-36 mg/kg). Prikazani podaci upućuju na dostatan unos joda u školske djece s područja Zagreba. Tireoglobulin je osjetljiv pokazatelj ne samo nedostatnog, nego i prekomjernog unosa joda u djece. Sadržaj joda u soli iz kućanstava školske djece bio je u skladu s hrvatskim zakonom o obveznom jodiranju soli (20-30 mg KI/kg soli).

Ključne riječi: *Jod – urin; Jod – unos; Jod – analiza; Dostatan unos joda; Suha kap krvi; Tiroglobulin – analiza; Štitnjača – funkcionalne pretrage; Štitnajača – ultrazvuk; Školska djeca*