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Publication Date

2017-07-01

DOI

10.1016/j.envres.2017.04.014

Peer reviewed

Title Page

Factors associated with serum thyroglobulin in a Ukrainian cohort exposed to iodine-131 from the accident at the Chernobyl Nuclear Plant

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Abstract

Background: Serum thyroglobulin (Tg) is associated with the presence of thyroid disease and has been proposed as a biomarker of iodine status. Few studies have examined factors related to serum Tg in populations environmentally exposed to ionizing radiation and living in regions with endemic mild-to-moderate iodine deficiency.

Methods: We screened 10,430 individuals who were living in Ukraine and under 18 years of age at the time of the 1986 Chernobyl Nuclear Power Plant accident for thyroid disease from 2001 to 2003. We estimated the percent change (PC) in serum Tg associated with demographic factors, iodine-131 thyroid dose, and indicators of thyroid structure and function using linear regression. We also examined these relationships for individuals with and without indications of thyroid abnormality.

Results: Mean and median serum Tg levels were higher among participants with abnormal thyroid structure/function. Percent change in serum Tg increased among females, smokers and with older age (p-values < 0.001), and Tg increased with increasing thyroid volume, and serum thyrotropin (p-values for trend < 0.001). We found no evidence of significant associations between iodine-131 thyroid dose and Tg. Serum Tg levels were inversely associated with iodized salt intake (PC= − 7.90, 95% confidence interval: − 12.08, − 3.52), and over the range of urinary iodine concentration, the odds of having elevated serum Tg showed a U-shaped curve with elevated Tg at low and high urinary iodine concentrations.

Conclusion: Serum Tg may be a useful indicator of population iodine status and a non-specific biomarker of structural and functional thyroid abnormalities in epidemiological studies.

Key words: thyroglobulin, ionizing radiation, iodine, thyroid, Chernobyl

Financial support: This research was supported by the Intramural Research Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services.

Human subjects research: Study subjects or accompanying guardians for minors provided written informed consent for the study. The study was evaluated and approved by the institutional review boards in Ukraine and the United States National Cancer Institute.

Abbreviations: ATg, antibodies to thyroglobulin; ATPO, antibodies to thyroid peroxidase; CI, confidence interval; FDR, false discovery rate; Gy, Gray; ^{131}I, iodine-131; PC, percent change; Tg, thyroglobulin; TSH, thyrotropin (or thyroid stimulating hormone); UIC, urinary iodine concentration

Publication history: The contents of this manuscript have never been published in any form.

Author Disclosure Statement: No competing financial interests exist.

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Manuscript

1. Introduction

Insufficient iodine intake in approximately 1.9 billion people worldwide is primarily due to environmental conditions, low levels of iodine in soils distant from coastal areas and in regions with frequent flooding (Ahad and Ganie, 2010). Iodine deficiency increases the risk of developing thyroid diseases (such as goiter, hypothyroidism and hyperthyroidism) and can result in impaired mental function and congenital abnormalities in children born to iodine deficient mothers (WHO et al., 2007). Despite the clinical importance of measuring iodine deficiency, commonly used indicators have a number of limitations in their ability to reliably assess current iodine status. Urinary iodine concentration (UIC) from spot urine reflects recent dietary iodine intake (hours to days) and can have high daily variability (Vejbjerg et al., 2009; Zimmermann et al., 2006). In contrast, high thyroid volume is an indicator of long-term iodine deficiency that may persist for years after iodine repletion (Zimmermann et al., 2006). Serum thyroglobulin (Tg), a thyroid protein that facilitates the uptake and storage of iodine in the thyroid gland, has been proposed as a biomarker of intermediate-term population iodine nutrition status (weeks to months) based on associations with UIC (Knudsen et al., 2001; Krejbjerg et al., 2016; Swanson et al., 2012; Vejbjerg et al., 2009), and sensitivity to changes in iodine status following iodine supplementation (Gordon et al., 2009; Ma and Skeaff, 2014; Skeaff and Lonsdale-Cooper, 2013; Vejbjerg et al., 2009; Zimmermann et al., 2006).

Most studies of serum Tg have been conducted among individuals with clinical manifestations of thyroid disease, such as subacute thyroiditis (Hidaka et al., 1994), Graves' disease (Izumi and Larsen, 1978), and thyrotoxicosis factitia (Mariotti et al., 1982), or among people with a history of thyroid cancer (Ericsson et al., 1984; Haugen et al., 2016; Heemstra et al., 2007; McGrath et al., 2015; Molinaro et al., 2013; Rosario et al., 2013; Trimboli et al., 2015). While several epidemiologic studies have examined factors related to serum Tg in large population-based settings (Krejbjerg et al., 2016; Ma and Skeaff,

2014), only one other study has examined factors related to Tg in a radiation exposed population with mild-to-moderate iodine deficiency (Cahoon et al., 2013). In addition, studies reporting serum Tg concentrations often fail to assess assay interference by serum autoantibodies (Spencer et al., 2011), and may use different measurement assays without proper inter-study assay standardization, complicating both quantification of serum Tg concentration and comparability to other studies (Demers and Spencer, 2002; Spencer et al., 1998).

The objective of this study is to examine demographic characteristics, indicators of thyroid structure and function, and other factors in relation to serum Tg concentration in the Ukrainian-American Cohort Study of Thyroid Cancer and Other Thyroid diseases (UkrAm), 15–17 years after the Chernobyl accident. This study population may be particularly susceptible to thyroid diseases due to environmental exposures to ^{131}I and endemic mild-to-moderate iodine deficiencies.

2. Materials and methods

2.1. Study population

Our study population includes 12,379 individuals in Ukraine who were exposed as children and adolescents (<18 years of age) to radiation from the Chernobyl accident on April 26, 1986 (Tronko et al., 2012). Measurements of serum Tg and antibodies to thyroglobulin (ATg) were made for individuals participating in the second biennial screening cycle (2001-2003), which was 15− 17 years after the accident. We excluded 57 individuals with a history of thyroid cancer, 64 with thyroid cancer at the 2nd cycle, 24 with follicular adenoma, 827 who reported a history of thyroid disease (nodular or diffuse goiter, thyroiditis, hyperthyroidism or hypothyroidism), 2 who reported thyroid surgery, 26 who reported intake of thyroid hormones prior to the 2nd cycle; and 1 who did not have a thyroid gland. To reduce possible assay interference, we excluded 673 subjects with serum ATg concentrations >60 IU/mL. We also excluded 236 subjects without serum Tg values and 39 with Tg equal to zero. Our final study sample

consisted of 10,430 subjects. Study subjects or accompanying guardians for minors provided written informed consent for the study. The study was evaluated and approved by the institutional review boards in Ukraine and the United States National Cancer Institute.

2.2. Screening examination

The details of the screening examination procedure have been previously described (Stezhko et al., 2004; Tronko et al., 2012). Screening consisted of ultrasonography with 7.5-MHz ultrasound probes and thyroid palpation by a trained ultrasonographer, and independent clinical examination and palpation by an endocrinologist. Blood and spot urine samples were collected to measure serum thyroid hormones and antibody concentrations, and urinary iodine concentration. Standardized questionnaires assessing sociodemographic characteristics, residential history, dietary and medical history, and thyroid dose estimation were administered by study personnel. Tg, thyrotropin (TSH), ATg, and anti-thyroid peroxidase (ATPO) concentrations were measured in serum samples with LUMItest immunochemiluminescence assays (Brahms Diagnostica GMBH, Heningsdorf, Germany) using a Berthold 953 luminometer (Berthold Technologies, GmbH & Co. KG, Bad Wildbad, Germany) (McConnell et al., 2007). Urinary iodine content was measured using the Sandell-Kolthoff reaction, as described by Dunn and colleagues (Dunn et al., 1993). Thyroid volume was calculated based on the volume of an ellipsoid, as described by Brunn and colleagues (Brunn et al., 1981).

2.3. Dosimetry

Detailed methods for ^{131}I thyroid dose reconstruction have been described by Likhtarov and colleagues (Likhtarov et al., 2014). Briefly, assessment of thyroid doses from ^{131}I was based on direct readings (readings of gamma radiation from radiation detectors placed on the neck), age- and sex-specific thyroid masses derived for the Ukrainian population from the results of ultrasound measurements done in 1991-

1996, questionnaires on residential history, dietary and lifestyle habits, and environmental transfer models. One thousand individual stochastic doses were calculated for each cohort member accounting for shared and unshared errors. The distribution of dose estimates was close to lognormal with geometric standard deviations (GSD) ranging from 1.6 to 5.4 among cohort members (Likhtarov et al., 2014). The arithmetic mean of 1000 individual stochastic dose realizations was used for this analysis. The arithmetic mean (geometric mean) for these individual ^{131}I stochastic doses was 0.67 (0.20) Gray (Gy) (Tronko et al., 2006). Only thyroid doses due to ^{131}I intake were considered in this study, as other exposure pathways (intake of short-lived ^{132}I and ^{133}I, external irradiation and ingestion of long-lived isotopes of cesium) typically account for no more than 5–10% of the total thyroid dose (Likhtarov et al., 2014).

2.4. Statistical analysis

In this exploratory analysis evaluating the relationship between factors associated with Tg and serum Tg concentration, linear regression analyses were used to compute change in log serum Tg and 95% confidence intervals (CIs). Based on previously associations with serum Tg concentration, we considered the following factors potential predictors of serum Tg in this study: sex, age at examination, calendar year of examination, age at exposure, oblast (an administrative subdivision similar to a state or province) of residence, urban or rural residence, smoking status, ^{131}I thyroid dose, serum TSH, ATg and ATPO, UIC, thyroid volume, ultrasound detected thyroid nodules, season, vitamin use, and self-reported intake of iodine rich foods (herring, fish other than herring, and seaweed), use of iodized salt, and iodine supplementation. Missing values for smoking, oblast, UIC and use of iodized salt were coded as separate categories and included as indicator variables in the models. Criteria for inclusion of covariates was based on forward selection with α=0.05. Variables that were initially excluded in forward selection model were evaluated individually for model influence. The variable was left in the model if any of the parameter estimates of the forward selection model changed by more than 10% after adding the variable to the

model. The final model included sex, age at examination (15− 18, 19− 22, 23− 26, 27− 34 years), calendar year at examination, smoking status, oblast and urban/rural residence at examination, thyroid volume quintiles, the presence of thyroid nodules, serum TSH (≤0.3 (the lower limit of the reference range), 0.4− 1.6, 1.7− 2.8, 2.9− 3.9, ≥4.0 (the upper limit of the reference range)) (Ostroumova et al., 2013), ATg tertiles and ATPO quartiles, ^{131}I thyroid doses (0.0003− 0.099, 0.10− 0.249, 0.25− 0.499, 0.50− 0.99, 1.0− 2.49, 2.5− 5.0, and >5.0 Gy) (Ostroumova et al., 2013), UIC (0.1− 20, 20− 49, 50− 99, 100− 199, 200− 299, and > 300 µg/L) (WHO et al., 2007), and use of iodized salt. We also assessed self-reported intake of iodine rich foods (herring, fish other than herring, and seaweed), and iodine supplementation. We natural log-transformed serum Tg; and the residuals of models using this dependent variable were approximately normally distributed after log-transformation. Kruskal-Wallis tests of heterogeneity of serum Tg medians for categorical descriptors, and tests for trends and chi-squared p-values for independent ordinal variables in linear regression models were performed.

We then examined associations between these predictive factors and ln[serum Tg] using linear regression analyses in which:

$$\ln[Tg_i] = \sum_{j=1}^{P} \beta_j X_{ij} + \varepsilon_i \qquad (1)$$

for some independent variables $(X_{ij})_{j=1}^{P}$. For ease of interpretation, the model coefficients, β_j, were converted to percent change (PC) –defined as follows:

$$\%\Delta Tg_j = 100(\exp(\beta_j)-1)$$

Percent change is a unitless measure that represents the change in the geometric mean of serum Tg comparing one category of a factor to the referent category after adjusting for all other factors. Serum Tg concentration was dichotomized to elevated Tg (>40 µg/L; n=920), which is the upper limit from the adult reference range of serum Tg concentration in an iodine replete American population (Demers and

Spencer, 2002) to estimate the odds ratio (OR) and 95% confidence interval (CI) of elevated serum Tg for UIC categories and thyroid volume quintiles. We also tested for departure from linearity (on a log scale) by including quadratic terms for continuous UIC and thyroid volume in multivariable linear regression models. In sensitivity analyses, we examined dose-response associations between quintiles of ^{131}I thyroid dose (< 0.05, 0.05– 0.13, 0.14– 0.28, 0.29– 0.73 and > 0.74 Gy), thyroid dose with ≥ 2.5 Gy as the highest category, and thyroid dose categories < 5 Gy and serum Tg. We tested for non-linear dose-response trends by including the continuous quadratic term for ^{131}I thyroid dose in the models.

We assessed whether the relationship of various factors and serum Tg differed between participants with normal and abnormal thyroid status. 'Abnormal' thyroid structure/function was defined as serum TSH levels ≤ 0.3 or > 4.0 mIU/L (based the TSH assay manufacturer's reference range), thyroid volume > 25 mL in males or > 18 mL in females (mean thyroid volume + 3 SD in iodine sufficient populations (Rasmussen et al., 2002)), ultrasound detected presence of thyroid nodules, or diagnosis of prevalent thyroid disease including presence of diffuse goiter on palpation or ultrasound. We tested for statistical interaction between predictors of serum Tg and normal/abnormal thyroid status using likelihood ratio tests in fully adjusted models. To test the proportion of variance in serum Tg concentration explained by the covariates, we calculated the R-squared for each of the multivariate linear regression models. Using receiver operating characteristics curve analysis for the diagnostic performance of serum Tg to predict normal/abnormal thyroid status, we calculated the area under the curve. We accounted for multiple comparisons by using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to correct the false discovery rate (FDR), defined as the expected proportion of erroneous rejections of the null hypothesis among the total number of rejected hypotheses. Tests were two sided and considered significant at α=0.05. Statistical analyses were performed using SAS v9.2 (SAS Institute, Cary, NC) and figures were created using Stata v13.1 (StataCorp LP, College Station, TX).

3. Results

Mean and median serum Tg concentrations for demographic characteristics and physiological markers of thyroid function for 10,430 subjects by normal/abnormal thyroid status are shown in Table 1. We observed significant heterogeneity in median serum Tg concentration across categories of attained age, year of examination, UIC, serum TSH, ATg and ATPO concentrations, thyroid volume, and ^{131}I thyroid dose, regardless of thyroid structure/function status. Median serum Tg concentrations were higher among the abnormal thyroid structure/function group compared to the normal thyroid group, for all factors (p-interaction < 0.0001, data not shown). Median serum Tg concentration increased with increasing serum TSH concentration, thyroid volume and ^{131}I thyroid dose, while median serum Tg concentration decreased with increasing UIC and serum antibody concentrations (p-trend < 0.01 for all factors).

Associations of demographic characteristics, UIC, ^{131}I thyroid dose, indicators of thyroid function and iodized salt consumption with serum Tg, adjusted for possible confounders are shown in Table 2. Females had 26% higher serum Tg concentration (95% CI: 20.91, 30.27) than males, and smokers had 18% higher serum Tg concentration (95% CI: 13.59, 22.90) than non-smokers. Serum Tg levels also differed by iodine status in both the normal and abnormal thyroid groups. Overall, individuals with adequate iodine intake (UIC ≥ 100 μg/L) had 20% lower serum Tg concentration (95% CI: − 25.02, − 15.43) compared to severely iodine deficient individuals (UIC < 20 μg/L). Similar to the unadjusted analyses, serum Tg levels increased with increasing TSH concentration (p-trend < 0.0001), and serum ATg showed inverse linear relationships with serum Tg concentration (p-trend < 0.0001) in adjusted models. Among indicators of dietary intake of iodine (i.e. iodine supplementation, use of iodized salt, and consumption of iodine-rich foods: seaweed, herring, or other ocean fish), only use of iodized salt was significantly associated with decreased serum Tg concentration (p-value = 0.0005) (Table 2). In sensitivity analyses, we did not observe linear dose-response relationships for ^{131}I thyroid dose and serum Tg using ^{131}I thyroid dose quintiles (p-value for trend = 0.5324), ≥ 2.5 Gy as the highest dose category (p-value for trend 0.3865), or

for thyroid doses < 5 Gy (p-value for trend = 0.4025). We also found no evidence of non-linear dose-responses for these dose categories (^{131}I thyroid dose^2 p-value for all > 0.50) (data not shown).

There was evidence of effect modification of serum Tg associations with thyroid volume by normal/abnormal thyroid status. Higher increases in serum Tg concentration were found with increasing thyroid volume among participants with abnormal thyroid structure/function (p-interaction=0.0180). All statistically significant values persisted after controlling for the false discovery rate, except for the interaction between attained age and normal/abnormal thyroid status (corrected p-value for interaction=0.2292).

We examined odds of elevated serum Tg by categories of iodine status (Fig. 1 and Supplemental Fig. 1) and by thyroid volume quintiles (Fig. 2). The graph of the odds of elevated serum Tg for iodine status categories showed a U-shaped curve in both adjusted (Fig. 1) and unadjusted (Supplemental Fig. 1) analyses. In multivariate linear regression, we also found statistically significant non-linear trends for associations between log serum Tg concentration and continuous UIC (p-value for UIC^2<0.001 for both adjusted and unadjusted analyses). The odds of elevated serum Tg increased across quintiles of thyroid volume, with a statistically significant non-linear trend for associations between log serum Tg and continuous thyroid volume (thyroid volume^2 p<0.0001).

4. Discussion

We examined factors associated with elevated serum Tg levels in a Ukrainian cohort exposed as children and adolescents to radiation from the Chernobyl accident. We found increased serum Tg concentration for female sex, higher attained age, cigarette smoking, increasing serum TSH, larger thyroid volume and the presence of thyroid nodules, as well as a U-shaped curve for the odds of elevated Tg across categories of iodine status.

4.1. Iodine status

Analysis of soil and water samples, diffuse euthyroid goiter prevalence, and UIC measurements suggest that residents of Ukraine exposed to radiation from the Chernobyl accident were historically iodine deficient, and remained mild-to-moderately iodine deficient despite iodine supplementation (Tronko et al., 2005). Previous studies have demonstrated inverse associations between serum Tg concentration and UIC over the range of iodine excretion (Bayram et al., 2009; Bilek et al., 2015; Buchinger et al., 1997; Cahoon et al., 2013; Knudsen et al., 2001; Krejbjerg et al., 2016; Rasmussen et al., 2002; Thomson et al., 2001; Vejbjerg et al., 2009; Zimmermann et al., 2013), and positive associations with long-term measures of iodine intake (such as, increased thyroid volume, and the presence of thyroid nodules, and prevalent goiter) in iodine deficient areas (Krejbjerg et al., 2016; Rasmussen et al., 2002; Thomson et al., 2001). We found consistent independent associations between serum Tg and multiple indicators of population iodine status (i.e., UIC, serum TSH and thyroid volume), suggesting that measuring serum Tg may capture both population iodine status and thyroid dysfunction due to iodine deficiency. However, we cannot rule out residual confounding, possible covariate measurement errors and unknown factors that may bias the associations between these indicators of iodine status and serum Tg. Assuming that the measurement errors are of classical form, we would expect our results to be biased towards the null (Carroll et al., 2006). Measuring serum Tg in addition to short-term indicators of iodine intake (e.g., UIC) and long-term indicators of iodine deficiency (e.g., thyroid volume and goiter) may therefore provide a more comprehensive assessment of population iodine status and thyroid function.

Previous studies have also reported serum Tg as a sensitive biomarker of iodine status, with decreased serum Tg levels following dietary iodine supplementation (Krejbjerg et al., 2016; Ma et al., 2016; Missler et al., 1994; Rasmussen et al., 2002; van den Briel et al., 2001; Vejbjerg et al., 2009). We reported significantly decreased serum Tg concentration among participants that consumed iodized salt. In

addition, the U-shaped association showing increased odds of elevated serum Tg for individuals with iodine deficiency and excess iodine intake in this study was similar to U-shaped curves for associations of serum Tg with UIC reported in a recent study of biomarkers of iodine deficiency and excess in 2512 children ages 6– 12 from 12 countries (Zimmermann et al., 2013), and a study of 1858 individuals ages 6– 98 from the Czech Republic (Bilek et al., 2015). The U-shaped associations between UIC and serum Tg suggest that serum Tg is a sensitive indicator of population iodine status at both low iodine intake and excess iodine intake (Zimmermann et al., 2013). Higher levels of serum Tg in subjects with iodine deficiency or too much iodine intake may be the result of increased Tg production by the thyroid due to TSH hyperstimulation, thyroid hyperplasia and increases in thyroid size (Zimmermann et al., 2013; Zimmermann et al., 2006). However, in our mostly iodine deficient study population, assessment of serum Tg and thyroid function at high levels of iodine excretion are limited because only 4% of the study population had more than adequate or excess iodine intake (UIC $\geq$ 200 μg/L).

Differences in serum Tg levels by calendar year also suggest sensitivity to changes in iodine intake. Tronko and colleagues reported that mean and median UIC increased from the 1st screening cycle (1998–2000) to the 2nd screening cycle (2001–2003) for all oblasts (Zhitomir, Kiev and Chernihiv) and in both urban and rural areas after the Ukrainian government implemented national and regional programs for the elimination of iodine deficiency in 2001 (Tronko et al., 2005). The significantly lower serum Tg for screenings in calendar years 2002 and 2003 compared to 2001 may therefore reflect the effectiveness of these interventions. Collectively, the closely tracked responses of serum Tg across the range of iodine intake and significant associations with iodized salt intake in this population demonstrate serum Tg as a biological indicator of population iodine status regardless of environmental radiation exposures.

4.2. Demographic factors, thyroid function and ^{131}I thyroid dose

Our results showed that female sex, attained age, cigarette smoking, serum TSH concentration, and thyroid volume were independently associated with increased serum Tg concentration. Previous studies have reported elevated serum Tg in females (Cahoon et al., 2013; Fenzi et al., 1985; Giovanella et al., 2011; Knudsen et al., 2001; Thomson et al., 2001; Vejbjerg et al., 2009) and with attained age (Cahoon et al., 2013; Knudsen et al., 2001; Vejbjerg et al., 2009), while Giovanelli et al. (2011) reported no difference in serum Tg by age in healthy individuals–suggesting that differences in serum Tg by age are due to thyroid dysfunction. We found higher serum Tg levels in females than males, and among older individuals in this study, which may be attributed to higher prevalence of thyroid diseases in these groups (despite adjusting for known thyroid abnormalities and dysfunction). We therefore cannot rule out residual confounding of these associations by unmeasured factors.

Several studies have shown cigarette smoking to be associated with increased serum Tg (Bertelsen and Hegedus, 1994; Cahoon et al., 2013; Knudsen et al., 2002; Krejbjerg et al., 2016), and with significant changes to indicators of thyroid dysfunction (including decreased serum TSH, increased thyroid size, and increased risk of prevalent non-toxic goiter and thyroid nodules in iodine deficient areas) (Knudsen et al., 2002; Pedersen et al., 2008; Wiersinga, 2013). Increases in serum Tg among smokers have been attributed to thiocyanate–a metabolite of the cigarette smoke component, cyanide, and a competitive inhibitor of thyroidal iodine uptake (Tonacchera et al., 2004). The resulting insufficient iodine uptake in the thyroid leads to thyroid cell hyperplasia and increased serum Tg production (Wiersinga, 2013). A few studies have reported correlations between serum TSH and serum Tg concentration in areas with severe iodine deficiency only (Lima et al., 1986; Missler et al., 1994), while other studies found no correlations across all TSH levels (Feldt-Rasmussen et al., 1979; Fenzi et al., 1985; Knudsen et al., 2001). Our findings of independent positive dose-response relationships of both serum TSH and thyroid volume with serum Tg concentration, suggest that increased thyroid mass and thyroid stimulation by TSH (Demers and Spencer, 2002) may contribute to elevated serum Tg in this mild-to-moderately iodine deficient cohort.

The higher serum Tg levels among rural residents compared to urban residents, and differences in serum Tg by oblast, may reflect regional differences in iodine deficiency based on soil and ground water iodine content, and access to iodine-rich foods and iodine supplementation (Tronko et al., 2005). The urban/rural differences in iodine status are consistent with previous studies of areas affected by the Chernobyl accident (Cahoon et al., 2013; Hatch et al., 2011; Tronko et al., 2005), and reflect the consumption of local food and water in rural areas, and that urban areas were likely to have greater access to iodine-rich foods and iodine intervention programs (e.g. iodized salt, multivitamins containing iodine) (Tronko et al., 2005). In addition, nitrate in drinking water and foods (primarily from the use of nitrogen fertilizers) may interfere with iodine uptake by the thyroid and has been associated with increased risks of thyroid dysfunction, goiter and thyroid cancer (Drozd et al., 2015; Ward et al., 2010). Exposures to high levels of nitrate in rural and agricultural areas may result in effects similar to iodine deficiency and contribute to the higher levels of serum Tg among rural residents (Drozd et al., 2015).

In this study, we found no evidence of statistically significant linear or non-linear dose-response relationships between ^{131}I thyroid dose and serum Tg, and most of the associations between categories of thyroid dose and serum Tg were not statistically significant and overlapped each other and zero. Similarly, previous studies of populations environmentally exposed to radiation have reported no evidence of significant associations of radiation dose with serum Tg (Cahoon et al., 2013; Morimoto et al., 1987).

4.3. Antibodies and interference

Our findings of inverse associations between serum ATg and serum Tg at levels below 60 IU/mL suggests possible assay interference by serum thyroid autoantibodies, which may lead to an underestimation of "true" serum Tg concentrations (Ahn et al., 2013; Spencer et al., 2011). The strong interference with serum Tg measurements at low ATg levels may be partially explained by ATg

heterogeneity and the differing epitope binding specificities of the various anti-Tg antibodies (Madureira et al., 2008; Spencer and Lopresti, 2008). In addition, ATg interference leading to an underestimation of Tg levels is common with immunometric assays, and there appears to be no threshold concentration for ATg at which interference with Tg does not occur (Clark and Franklyn, 2012; Spencer et al., 2011; Spencer, 2000).

4.4. Strengths and limitations

Strengths of our study include the large sample size (over 10,000 subjects), availability of data on many sociodemographic factors, standardized clinical examinations, and measurement of biochemical indicators of thyroid function and iodine status using sensitive assays. In addition, possible serum Tg assay interference was largely controlled for by concurrent measurements of serum Tg and ATg. However, this study was cross-sectional with only a single measurement of thyroid hormones and urinary iodine concentration, which did not allow assessment of temporal associations between the predictors and serum Tg, or intrapersonal variability in serum Tg. We also cannot rule out residual confounding by measured and unmeasured factors. For example, smoking status was assessed using a dichotomous variable (current smoker/non-smoker), which does not capture smoking frequency, intensity, or duration, and may result in residual confounding. We accounted for possible serum Tg assay interference from ATg by excluding individuals with ATg levels above 60 IU/mL, and adjusted our multivariate analyses by including ATg in the regression models. However, using a cutoff of 60 IU/mL may not have adequately accounted for assay interference at lower thyroid antibody levels or manifestations of thyroid disease attributed to thyroid autoantibodies.

5. Conclusions

Our results support a growing literature demonstrating serum Tg as an indicator of population iodine status and thyroid dysfunction, and expand findings to populations living in regions with endemic iodine deficiency and environmentally exposed to radiation.

Tables and Figures

Table 1. Factors associated with serum Tg, stratified by normal and abnormal structure/function

	All (n=10,430)				Normal thyroid (n=7,880)				Abnormal thyroid[a] (n=2,550)			
	n	Mean Tg	Median Tg	P-value[b,c]	n	Mean Tg	Median Tg	P-value[b,d]	n	Mean Tg	Median Tg	P-value[b,e]
Sex[f]												
Male	5517	18.07	12.4	0.0007	4070	15.89	11.2	0.0002	1447	24.21	16.5	0.0631
Female	4913	19.28	13.0		3810	16.89	12.0		1103	27.54	16.9	
Age at examination, year[f,g]												
15-18	2478	19.81	13.4	< 0.0001	1946	17.61	12.5	< 0.0001	532	27.82	16.8	< 0.0001
19-22	2455	16.37	10.7		1863	14.45	9.9		592	22.43	13.7	
23-26	2530	19.28	13.1		1936	16.92	12.0		594	26.97	17.4	
27-34	2967	19.01	13.3		2135	16.43	11.9		832	25.63	18.4	
Calendar year at examination[f,g]												
2001	4587	23.96	16.6	< 0.0001	3540	20.77	15.1	< 0.0001	1047	34.74	22.9	< 0.0001
2002	5101	14.55	10.5		3806	12.82	9.5		1295	19.62	13.8	
2003	742	13.93	10.0		534	12.55	9.1		208	17.49	13.0	
Oblast/urban or rural residence at examination[f,g]												
Zhitomir urban	463	15.83	10.9	< 0.0001	391	14.12	10.2	< 0.0001	72	25.15	14.9	< 0.0001
Zhitomir rural	2301	29.39	20.4		1598	25.88	18.9		703	37.37	24.7	
Kiev/Kiev City urban	1140	12.02	8.9		936	11.49	8.7		204	14.41	10.5	
Kiev/Kiev City rural	1061	15.27	11.2		824	13.65	10.3		237	20.93	15.4	
Chernihiv urban	1559	13.05	9.4		1280	11.99	9.1		279	17.90	13.1	
Chernihiv rural	3881	17.73	13.2		2828	15.73	12.1		1053	23.11	16.5	
Missing	25	17.69	8.6		23	14.64	8.6		2	52.75	52.8	
Smoking status[f]												
Non-smoker	6973	18.49	12.3	0.0005	5363	16.21	11.4	0.0023	1610	26.12	16.5	0.8024
Current smoker	3456	18.94	13.4		2516	16.73	12.3		940	24.86	17.3	
Missing	1	13.90	13.9		1	13.90	13.9					
UIC, μg/L[f,g]												
0.1-19	1268	22.62	16.6	< 0.0001	940	19.85	14.7	< 0.0001	328	30.55	21.7	< 0.0001
20-49	3795	19.89	13.7		2818	17.73	12.5		977	26.11	17.6	
50-99	3237	17.32	11.9		2473	15.46	11.1		764	23.36	15.8	
100-1014	1813	15.35	9.7		1404	12.69	9.0		409	24.50	12.9	
Missing	317	20.16	14.6		245	17.81	13.3		72	28.15	19.8	
Serum TSH, mIU/L[f,g]												
0-0.3	95	15.82	10.9	< 0.0001					95	15.82	10.9	< 0.0001
0.4-1.6	6195	16.89	11.8		4705	14.96	10.8	< 0.0001	1490	23.00	15.4	
1.7-2.8	3240	19.95	13.6		2615	17.76	12.6		625	29.13	20.4	
2.9-3.9	664	25.28	16.8		560	21.83	15.2		104	43.84	26.4	
4.0-44.3	236	29.15	17.5						236	29.15	17.5	
Serum ATPO, IU/mL[f,g]												
0-10	2704	18.61	12.6	< 0.0001	2083	16.42	11.4	0.0030	621	25.95	16.5	0.0204
11-19	2683	18.65	13.0		2025	16.79	11.9		658	24.37	17.5	
20-29	2483	19.44	13.1		1862	16.98	12.0		621	26.83	17.9	
30-11,887	2560	17.89	11.9		1910	15.29	11.0		650	25.55	15.1	
Serum ATg, IU/mL[f,g]												
0-13	3599	19.87	13.5	< 0.0001	2759	17.49	12.5	< 0.0001	840	27.67	18.3	0.0004
14-25	3435	18.49	12.9		2611	16.35	11.8		824	25.25	16.8	
26-60	3396	17.50	11.6		2510	15.16	10.5		886	24.13	15.2	
^{131}I thyroid dose, Gy[f,g]												
0.0003-0.099	3555	15.68	11.3	< 0.0001	2753	14.14	10.4	< 0.0001	802	20.99	14.8	< 0.0001
0.10-0.249	2435	17.97	12.1		1809	15.60	10.8		626	24.81	16.0	
0.25-0.49	1616	19.70	13.6		1198	17.04	12.2		418	27.34	18.8	

0.50-0.99	1269	19.74	13.4		960	17.42	12.6		309	26.95	17.5	
1.00-2.49	1026	24.35	16.7		766	21.36	15.1		260	33.16	19.6	
2.50-4.99	339	23.69	15.3		248	21.77	15.1		91	29.17	15.8	
5.00-41.6	186	26.36	15.3		143	20.20	14.6		43	46.82	21.0	
Missing	4	21.78	19.6		3	26.40	22.7		1	7.90	7.9	
Thyroid volume, mL[f,g]												
1.8-7.9	2124	14.55	10.6	< 0.0001	2008	14.41	10.4	< 0.0001	116	17.09	12.0	< 0.0001
8.0-9.6	2025	15.92	10.9		1905	16.01	11.0		120	14.59	10.4	
9.7-11.6	2132	17.20	12.2		1999	16.66	11.9		133	25.43	14.8	
11.7-14.9	2066	19.61	13.6		1645	18.14	13.4		421	25.35	15.0	
15.0-88.8	2083	25.97	17.4		323	20.02	13.8		1760	27.06	18.0	
Presence of ultrasound detected nodules at screening[f]												
No	10032	18.35	12.5	< 0.0001	7880	16.37	11.6		2152	25.6	16.8	0.8024
Yes	398	25.97	16.5						398	25.97	16.5	
Use of iodized salt[f]												
No	8790	19.36	13.1	< 0.0001	6624	16.98	12.0	< 0.0001	2166	26.65	17.3	< 0.0001
Yes	1599	14.44	10.0		1222	12.74	9.2		377	19.95	13.6	
Missing	41	27.70	23.7		34	28.11	23.6		7	25.71	27.5	

[a]Abnormal thyroid criteria: thyroid volume > 25 mL for males, thyroid volume > 18 mL for females, presence of thyroid disease or ultrasound-detected thyroid nodules, TSH < 0.3 mIU/L, or TSH ≥ 4.0 mIU/L.
[b]P-values from Kruskal-Wallis test of heterogeneity of medians
[c]All: p-values for trend are 0.0965 for age at examination, 0.0061 for serum ATPO, and <0.0001 for year at examination, UIC, serum TSH, serum ATg, ^{131}I thyroid dose and thyroid volume.
[d]Normal thyroid group: p-values for trend are 0.9367 for age at examination, 0.0265 for serum ATPO, and <0.0001 for year at examination, UIC, serum TSH, serum ATg, ^{131}I thyroid dose and thyroid volume.
[e]Abnormal thyroid group: p-values for trend are 0.1929 for age at examination, 0.0184 for serum ATPO, and <0.0001 for year at examination, UIC, serum TSH, serum ATg, ^{131}I thyroid dose and thyroid volume.
[f]P-values and p-trend values were adjusted for multiple comparisons using the false discovery rate.
[g]P-values from test for trend using chi-squared analyses in multivariate linear regression models.

Table 2. Percent change in Tg and 95% confidence intervals, stratified by normal and abnormal thyroid structure/function[a]

	All[b]		Normal Thyroid[c]		Abnormal Thyroid[d,e]		
Parameter	**PC (95% CI)[f]**	**p-trend[g]**	**PC (95% CI)[f]**	**p-trend[g]**	**PC (95% CI)[f]**	**p-trend[g]**	**$P_{interaction}$[h]**
Sex							
Male	ref		ref		ref		0.5753
Female	25.50 (20.91, 30.27)		25.81(20.68, 31.16)		22.77 (13.09, 33.28)		
Age at examination							
15-18	ref	< 0.0001	ref	0.0032	ref	< 0.0001	0.0382
19-22	-7.52 (-11.96, -2.86)		-8.24 (-13.14, -3.07)		-3.90 (-13.85, 7.19)		
23-26	2.56 (-2.56, 7.94)		0.49 (-5.06, 6.36)		11.37 (-0.82, 25.04)		
27-34	8.12 (2.73, 13.80)		5.17 (-0.71, 11.41)		19.54 (7.01, 33.53)		
Calendar year at examination							
2001	ref	< 0.0001	ref	0.0001	ref	< 0.0001	0.1419
2002	-29.53 (-32.12, -26.85)		-27.71 (-30.68, -24.61)		-35.00 (-40.07, -29.50)		
2003	-27.82 (-32.60, -22.71)		-25.48 (-31.11, -19.38)		-35.06 (-43.48, -25.38)		
Oblast/urban or rural residence at examination							
Zhitomir urban	ref		ref		ref		0.4476
Zhitomir rural	50.14 (37.95, 63.41)		55.20 (41.57, 70.15)		27.57 (2.59, 58.63)		
Kiev urban	-19.94 (-26.88, -12.34)		-16.97 (-24.63, -8.53)		-33.34 (-47.67, -15.09)		
Kiev rural	-2.08 (-10.73, 7.42)		-1.66 (-11.01, 8.66)		-9.36 (-28.57, 15.02)		
Chernihiv urban	-11.89 (-19.41, -3.67)		-10.79 (-18.91, -1.85)		-19.28 (-36.28, 2.26)		
Chernihiv rural	7.00 (-1.53, 16.26)		8.94 (-0.37, 19.12)		-5.36 (-23.86, 17.64)		
Smoking status							
Non-smoker	ref		ref		ref		0.8053
Current smoker	18.16 (13.59, 22.90)		19.57 (14.38, 25.00)		13.92 (4.70, 23.95)		
UIC, µg/L							
0.1-19	ref	< 0.0001	ref	< 0.0001	ref	0.0029	0.1479
20-49	-11.19 (-15.70, -6.44)		-10.17 (-15.33, -4.68)		-14.52 (-23.29, -4.75)		
50-99	-14.14 (-18.63, -9.42)		-12.38 (-17.54, -6.89)		-18.94 (-27.59, -9.25)		
100-1014	-20.37 (-25.02, -15.43)		-20.56 (-25.76, -14.99)		-18.55 (-28.40, -7.34)		
Serum TSH, mIU/L							
0.1-0.3	-24.41 (-36.39, -10.17)				-17.63 (-31.88, -0.41)		
0.4-1.6	ref	< 0.0001	ref	< 0.0001	ref	< 0.0001	0.1785
1.7-2.8	14.46 (10.46, 18.61)		13.35 (9.00, 17.87)		18.87 (9.37, 29.19)		
2.9-3.9	30.16 (21.69, 39.23)		25.02 (16.32, 34.37)		57.67 (32.34, 87.84)		
4.0-44.3	31.68 (16.57, 48.75)				52.12 (30.40, 77.47)		
Serum ATPO, mIU/L							
0-10	ref	0.9369	ref	0.9310	ref	0.8029	0.8271
11-19	3.04 (-1.45, 7.72)		3.77 (-1.28, 9.07)		1.611 (-7.75, 11.92)		
20-29	7.68 (2.86, 12.72)		7.61 (2.23, 13.28)		7.96 (-2.22, 19.21)		
30-11,887	-1.92 (-6.42, 2.79)		-1.33 (-6.41, 4.01)		-3.37 (-12.66, 6.90)		
Serum ATg, IU/mL							
0-13	ref	< 0.0001	ref	< 0.0001	ref	0.0030	0.5599
14-25	-4.09 (-7.77, -0.27)		-3.09 (-7.26, 1.26)		-7.85 (-15.36, 0.32)		
26-60	-10.03 (-13.63, -6.27)		-9.41 (-13.51, -5.11)		-12.76 (-20.00, -4.87)		
^{131}I thyroid dose, Gy							
0.0003-0.099	ref	0.1973	ref	0.2679	ref	0.6589	0.7807
0.10-0.249	3.03 (-1.38, 7.64)		2.76 (-2.19, 7.96)		3.25 (-5.92, 13.32)		
0.25-0.49	3.44 (-1.75, 8.92)		3.27 (-2.58, 9.48)		3.93 (-6.80, 15.91)		
0.50-0.99	2.32 (-3.35, 8.32)		1.52 (-4.81, 8.26)		4.50 (-7.46, 18.00)		
1.00-2.49	7.02 (0.44, 14.02)		5.65 (-1.73, 13.59)		10.25 (-3.29, 25.68)		
2.50-4.99	2.99 (-6.67, 13.64)		6.41 (-4.97, 19.15)		-5.79 (-22.78, 14.94)		
5.00-41.6	-0.81 (-12.82, 12.86)		-0.10 (-13.53, 15.43)		-6.48 (-29.58, 24.21)		
Thyroid volume, mL							

1.8-7.9	ref	< 0.0001	ref	< 0.0001	ref	< 0.0001	0.0015
8.0-9.6	11.67 (6.10, 17.53)		12.35 (6.70, 18.29)		2.37 (-18.59, 28.72)		
9.7-11.6	24.31 (18.12, 30.83)		23.21 (17.00, 29.75)		44.66 (15.50, 81.19)		
11.7-14.9	32.48 (25.57, 39.78)		31.61 (24.42, 39.22)		56.74 (28.35, 91.40)		
15.0-88.8	60.06 (48.48, 72.53)		45.47 (31.80, 60.54)		95.65 (60.36, 138.71)		
Presence of ultrasound detected nodules at screening							
No	ref				ref		
Yes	16.94 (6.47, 28.43)				24.20 (11.56, 38.28)		
Use of iodized salt							
No	ref		ref		ref		0.6376
Yes	-7.90 (-12.08, -3.52)		-9.07 (-13.69, -4.20)		-3.71 (-12.90, 6.45)		

Abbreviations: PC, percent change; CI, confidence interval; Ref, reference

[a]Area under the receiver operating characteristic curve for the model predicting normal/abnormal thyroid status from serum Tg = 0.62.

[b]R-squared for the model with all subjects = 0.15, $p < 0.001$.

[c]R-squared for the normal thyroid model = 0.12, $p < 0.001$.

[d]R-squared for the abnormal thyroid model = 0.16, $p < 0.001$.

[e]Abnormal thyroid criteria: thyroid volume > 25 mL for males, thyroid volume >18 mL for females, presence of ultrasound-detected thyroid nodules, TSH < 0.3 mIU/L, TSH ≥ 4.0 mIU/L, or presence of thyroid disease.

[f]Adjusted for all variables in the table.

[g]Test for trend p-value from likelihood ratio test for significance of adding corresponding variables to the model; p-trend values were adjusted for multiple comparisons using the false discovery rate.

[h]False discovery rate-adjusted p-values for interaction are 0.2292 for age at examination, 0.0180 for thyroid volume, 0.4282 for year at examination, UIC and serum TSH, and 0.8271 for sex, oblast, smoking, serum ATPO, serum ATg, ^{131}I thyroid dose, and use of iodized salt.

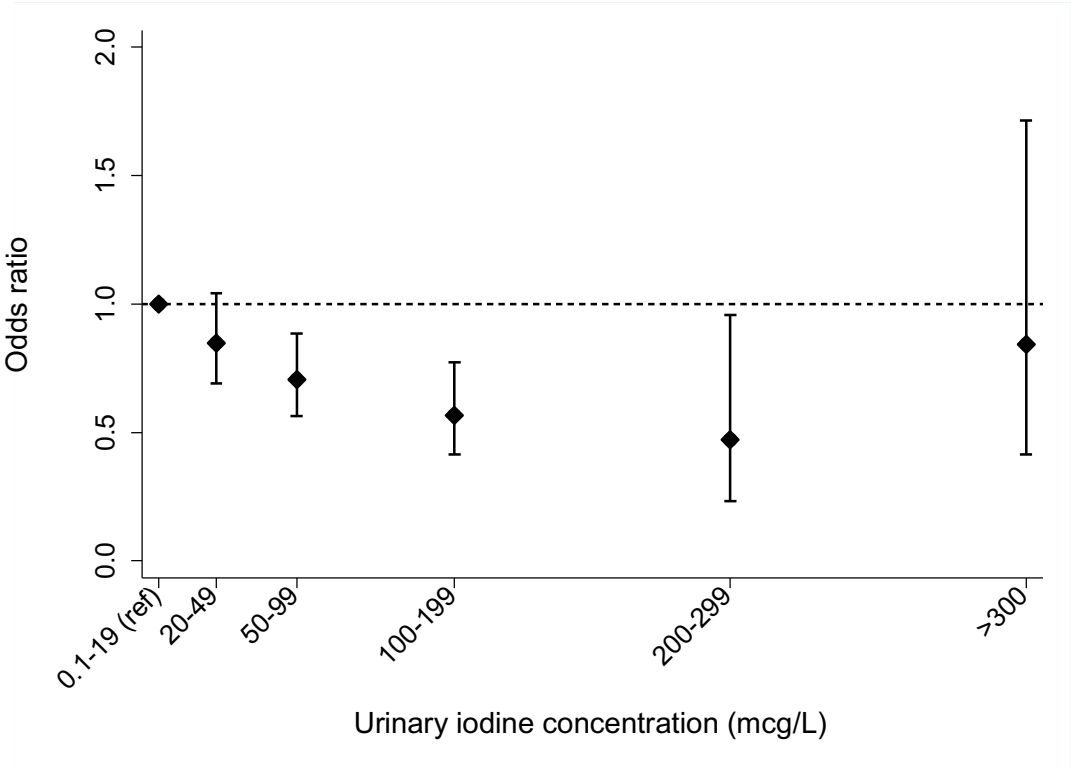

Fig. 1. Odds ratio with corresponding 95% CI of elevated serum Tg concentration (> 40 µg/L) by category of UIC. Urinary iodine concentration was categorized according to the WHO guidelines for dietary iodine intake: severely deficient (< 20 µg/L), moderately deficient (20–49 µg/L), mildly deficient (50–99 µg/L), and adequate iodine (100–199 µg/L), more than adequate (200–299 µg/L) and excessive iodine intake (> 300 µg/L). Adjusted for sex, age at examination, year of examination, oblast/urbanicity, smoking status, ^{131}I thyroid dose, serum TSH, serum ATPO, serum ATg, thyroid volume, ultrasound-detected thyroid nodules and use of iodized salt.

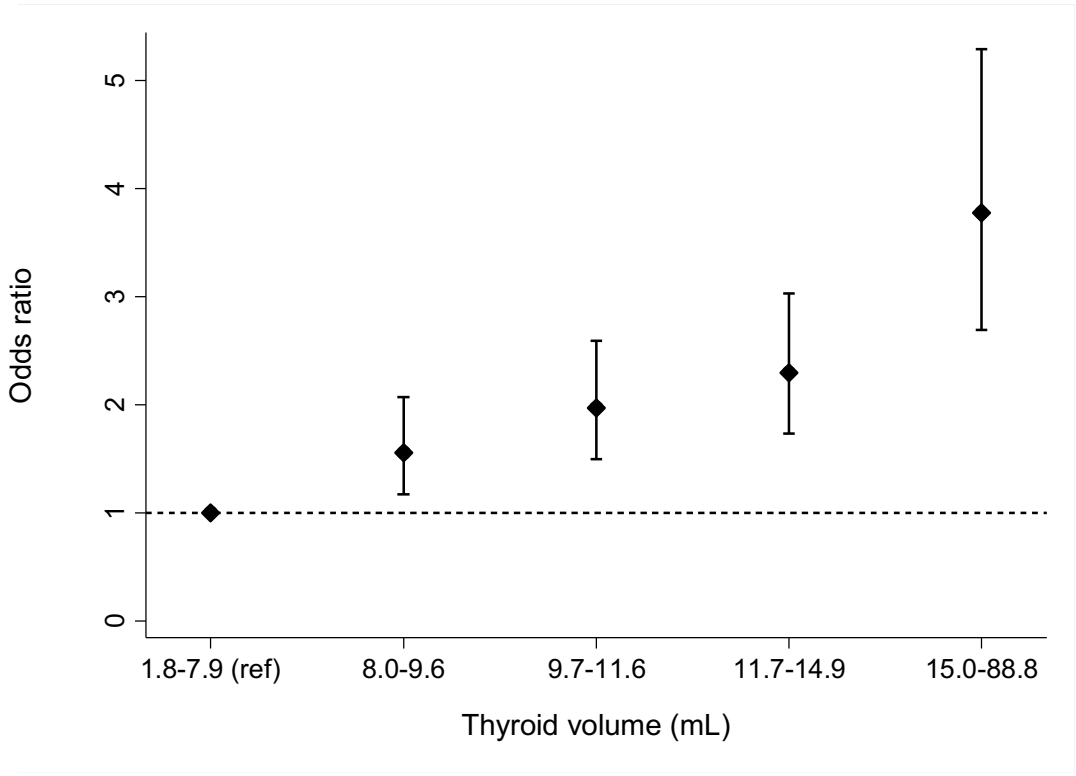

Fig. 2. Odds ratio with corresponding 95% CI of elevated serum Tg concentration (> 40 µg/L) by thyroid volume quintiles. Adjusted for sex, age at examination, year of examination, oblast/urbanicity, smoking status, UIC, ^{131}I thyroid dose, serum TSH, serum ATPO, serum ATg, ultrasound-detected thyroid nodules and use of iodized salt.

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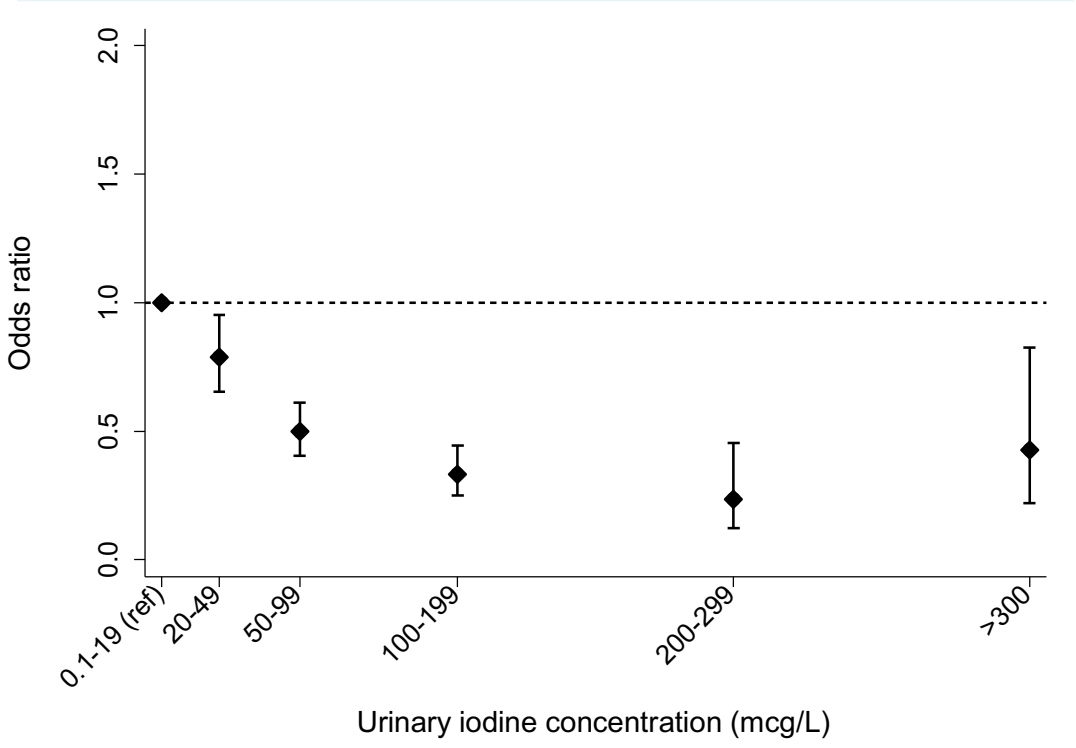
Supplemental data



Supplemental Figure 1. Unadjusted odds ratio with corresponding 95% CI of elevated serum Tg concentration (>40 µg/L) by category of UIC. Urinary iodine concentration was categorized according to the WHO guidelines for dietary iodine intake: severely deficient (<20 µg/L), moderately deficient (20-49 µg/L), mildly deficient (50-99 µg/L), and adequate iodine (100-199 µg/L), more than adequate (200-299 µg/L) and excessive iodine intake (>300 µg/L).