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Iodine status and thyroid nodules in females: a comparison of Cyprus and Romania



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ABSTRACT

Objectives: The increased comparative prevalence rates of thyroid cancer in Cyprus (>EU average) led us to conduct this study on possible risk factors of thyroid nodules. Romania served as a reference with a comparative thyroid cancer prevalence < EU average. This study aimed to assess the association between urinary iodine (UI) and thyroid nodules in adult females ($n = 208$) from Cyprus and Romania.

Study design: A case-control study ($n = 208$).

Methods: Cases were females with ultrasound-confirmed thyroid nodules and controls with confirmed absence of nodules. In both countries, subjects underwent ultrasound medical examinations, completed a questionnaire and offered a spot urine sample.

Results: Median UI level in Cyprus was 94 $\mu\text{g/L}$, whereas 32% of the Cypriot UI was < 50 $\mu\text{g/L}$, classifying the population as mildly iodine deficient. In Romania, both cases and controls were iodine sufficient. No significant differences ($P > 0.05$) in serum free thyroxine (fT4) and thyroid stimulating hormone (TSH) levels were found between cases and controls. Cases had lower median TSH levels compared with controls (1.4 mIU/L and 1.7 mIU/L, $P = 0.060$), but serum TSH and free thyroxine levels were within normal range. Albeit non-significant, participants with inadequate UI (<100 $\mu\text{g/L}$) had increased risk for thyroid nodules (odds ratio = 1.40, 95% confidence interval = 0.70, 2.81, $P = 0.346$), using multiple logistic regression after adjusting for age, body mass index, education, country and serum TSH.

Conclusions: This was the first study to quantify UI levels in Cyprus. While the Romanian iodine fortification programme reflected onto its UI levels, a representative assessment of iodine status in Cyprus will address the necessity of an iodine fortification programme.

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Abbreviations: TND, Thyroid nodular disease; UI, Urinary iodine; TSH, Thyroid stimulating hormone; fT4, Free thyroxine.

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Introduction

New incidents of thyroid cancer are steadily on the rise in Cyprus, since the last decade.¹ In 2012, thyroid cancer in Cyprus ranked third among females in the list of the most frequent cancer types with an age standardized incidence rate of 16.7 per 100,000,² whereas in Romania, thyroid cancer ranked 12th with 5.3 cases per 100,000.³ Improved diagnostics methods and thyroid screening activities could partially explain the observed increase in the incidence rates of both thyroid nodules^{4,5} and cancer.^{1,6,7} Risk factors for the development of thyroid nodules are: i) gender (females),^{8–10} ii) age^{8–12} and iii) family history of thyroid disorders.¹³ Emerging risk factors of thyroid nodules such as exposures to thyroid disrupting chemicals (like bisphenol A, perfluoroalkyl acids) remain to be explained.^{14,15} There is also evidence that inadequate iodine status is a risk factor for the development of thyroid nodules.¹⁶

Universal salt iodization is an effective measure to fight iodine deficiency disorders.¹⁷ In Romania, salt fortification is mandatory,¹⁸ whereas in Cyprus, there is no such national iodine fortification program. The association between urinary iodine (UI) measurements and thyroid nodules has been documented in cross-sectional studies conducted in different regions in China.^{9,19,20} Increased odds of thyroid nodules were observed for individual deficient or excessive UI levels (deficient: odds ratio [OR] = 2.97, 95% confidence interval [CI] 2.05 to 4.32 and excessive: OR = 1.87, 95% CI 1.29 to 2.24, $n = 2147$) with median UI levels 62 $\mu\text{g/L}$, 229 $\mu\text{g/L}$, and 750 $\mu\text{g/L}$ for the iodine deficient, sufficient and excessive groups, respectively.⁹ Chen et al.¹⁹ (2013) reported significant association only for UI levels <100 $\mu\text{g/L}$ and especially for females ($n = 9412$). On the other hand, an assessment of two cohorts in Denmark and a cross-sectional assessment of a study population in Germany demonstrated the lack of association between insufficient UI status (median UI < 100 $\mu\text{g/L}$) and thyroid nodules.^{21–23}

The risk factors associated with the thyroid nodules in either Cyprus or Romania have never been comprehensively investigated. This study aimed at determining the association between UI levels and thyroid nodular disease in adult females.

Methods

Study design

A pilot case-control study was set up, comprising of 57 cases and 65 controls in Cyprus, and 51 cases and 41 controls in Romania. Participants underwent medical examination at Archbishop Makarios III Hospital, in Nicosia, Cyprus and at the 'Ion Chiricuta' Institute of Oncology Cluj-Napoca (IOCN), Romania. Eligible participants were females >18 years, non-pregnant, not receiving any treatment for thyroid disorders. Cases were women having thyroid nodules of diameter >3 mm diagnosed by ultrasound. Controls were healthy women without thyroid nodules after ultrasound confirmation.

In Cyprus, controls were randomly recruited from the general population of Nicosia using phone and e-mail catalogues of the public sector. A total of 11% of approached individuals matched the eligibility criteria and were available for an appointment at the hospital. In Romania, controls that met the eligibility criteria were recruited from the nearby university campus and hospitals. Out of 51 cases and 41 controls recruited in Romania, two cases and three controls were excluded due to insufficient urine volume, whereas one participant was excluded from the total of 57 cases and 65 controls in Cyprus, due to indiscernible sample vial labelling.

Participants were interviewed by phone in Cyprus and with personal interviews in Romania. All responses to the questionnaires were translated to English, formatted and digitized with *EpiData Manager*.²⁴ Data harmonization was accomplished during the digitization of questionnaires, followed by both countries. The study was approved by the bioethics committee of the Republic of Cyprus (EEBK/EP/2013/27) and the ethics committee responsible for the development and quality assurance in clinical studies of the IOCN, Cluj-Napoca, Romania (decision no 16/25.06.2014).

Samples collection and analyses

All study participants were asked to provide a spot urine sample collected during their hospital visit. Serum hormone levels for thyroid stimulating hormone (TSH) and free thyroxine (fT4) were analysed for all the participants. In Cyprus, the inclusion of antibody measurements was decided at a later stage of the study, when the participant recruitment started in Romania and therefore antibody levels were analysed for a subsample only. Blood analyses were conducted at the laboratories of the Nicosia General Hospital using IMMULITE 1000 Siemens immunoassay system (Siemens Healthcare Diagnostics, ILL 60015-0778, USA, Abbott kit) and with Electrochemiluminescence (ECLIA; Cobas 6000, Roche kit) at the IOCN in Romania. Ultrasound assessment of the thyroid gland was performed by a single ultrasonographer in each country.

Spot urine samples were analysed for total iodine following the CDC Environmental Health protocol ITU004A²⁵ using inductively coupled plasma mass spectrometer (ICP-MS; Thermo X Series II, Thermo Scientific). The average recovery of spiked QCs was $95 \pm 5\%$ and $120 \pm 11\%$ in synthetic and pooled human urine, respectively. Urinary creatinine was determined by the picric acid-based spectrophotometric Jaffe-method.²⁶

Statistical analyses

Data analysis was done using R (version 3.2.2)²⁷ with the packages 'ggplot2',²⁸ 'Epi',²⁹ 'ResourceSelection',³⁰ 'car',³¹ and 'psych'.³² The pooled sample from both the countries, and the country-specific data were used. Non-normal distributed data were transformed to the natural logarithm. The t-test and Chi-squared tests were used to test for significant between-group differences. Univariate and multivariate logistic regression models were constructed to identify predictors of thyroid nodules and to assess the impact of iodine status on thyroid

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