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CLINICAL STUDIES

# Determination of normal human intrathyroidal iodine in Caracas population

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## Abstract

This study focuses on the determination of iodine content in healthy thyroid samples on male population from Caracas, Venezuela. Contribution to establish a baseline of iodine content in thyroid glands and hence to compare the iodine thyroid concentration of the Venezuelan population with other countries is also our objective. Male post-mortem individual samples were analyzed using a spectrophotometric flow injection method, based on the Sandell–Kolthoff reaction. The median intrathyroidal iodine concentration was $1443 \pm 677\,\mu g/g$ (wet weight), ranging from 419 to $3430\,\mu g/g$, which corresponds to a median of total iodine content of $15 \pm 8$ mg (ranging from 4 to 37). These results were higher than those values reported in the literature. No correlation of iodine content with age or weight of the healthy gland was found.


***Keywords:*** Total iodine; Thyroid gland; Sandell–Kolthoff reaction; Flow injection analysis; Intrathyroidal iodine

## Introduction

Iodine is an essential component of thyroid hormones. It is well known that changes in this element intake can affect the normal function of the gland because of the hormone synthesis alteration within the cells. The excess of intrathyroidal iodine may originate goiter, hypothyroidism and hyperthyroidism. On the other hand, deficiency of iodine may possibly result in endemic goiter, reduction in fertility, increase of prenatal and infant mortality, poor physical development, mental retardation and cretinism [1,2] and could have harmful effects on the cardiovascular system [3]. The lowest intrathyroidal iodine value has been reported in patients with thyroid gland cancer diseases [4–9].

Reports concerning iodine content on healthy human thyroid glands are limited. In general, these reports show a wide range of intrathyroidal iodine concentrations that mostly depends on the geographical location

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of the population [4,9–14]. Moreover, there is a significant correlation between the total iodine content and the size of the gland. Tiran [11] and Murillo [15] reported statistically significant differences on the iodine content of the thyroid gland section analyzed (lobes or nodule).

From a technical point of view, iodine has been determined in thyroid using the neutron activation analysis (NAA) [5–8,12,13], X-ray fluorescent analysis (XRF) [4,9,13,14] and the spectrophotometric method [15]. The latter is based on the Sandell and Kolthoff reaction [16] and offers some analytical advantages such as good sensitivity, selectivity and reproducibility, and it has the advantage that can be easily implemented for routine analysis in clinical laboratories. In this context, the main goal of the present work was to investigate the intrathyroidal iodine level in healthy thyroid glands from people who suffered an unexpected death in Caracas, Venezuela.

## Material and methods

### Instrumentation

The UV/VIS Shimadzu, Model UV150 spectrophotometer (Kyoto, Japan) having a 10 mm optical path length flow cell was used for all absorption measurements. The signal from the phototube detector was obtained and processed with an EZChrom Chromatography Data System version 6.8 (Scientific Software, Inc., USA). Fig. 1 presents the configuration of the flow injection (FI) system [17]. An Ismatec, Model IP peristaltic pump and a Rheodyne six-port injection valve were used for all solution deliveries. The reaction coil was inserted in a homemade oven. The temperature was fixed by a temperature controller (0.35 mm and 14.03 Ω/m ferromagnetic coil coupled to a 1/16 DIN microprocessor-based temperature controller, model 93AA1CK000RG, Series 93, WATLOW, MO, USA). This device is a better controller than the traditional thermostatic bath because it shows temperature fluctuations lower than 0.1 °C.

### Reagents and stock solutions

All chemicals that were used in this work were of AR grade. Distilled de-ionized water (DDW) was obtained from the Barnstead Nanopure System (Barsntead/thermolyne, Dubuque, IA, USA). A 1000 μg/mL stock solution of iodine was prepared daily by dissolving 0.1308 g of potassium iodine (Merk, Darmstadt, Germany) in water and making up to 100 mL in a volumetric flask. A solution of 0.008 M ammonium ceric (IV) sulfate was prepared by dissolving 5.06 g of $Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$ (Sigma-Aldrich, Steinheim, Germany) in 500 mL of 1.75 M $H_2SO_4$ (Riedel-de Haen, Seelze, Germany) solution and then diluting it to 1 L with DDW. Arsenious acid (0.1 M) was prepared by dissolving 10 g $As_2O_3$ (BDH, Poole, England) in 200 mL of 1 M NaOH (Merk, Darmstadt, Germany), then, 500 mL of 0.9 M $H_2SO_4$ and 40 g of NaCl (JT Baker, Phillipsburg, NJ, USA) were added, and finally diluted to 1 L with DDW. Finally, the alkaline digestion was accomplished by using 25% (w/v) of tetramethylammonium hydroxide (TMAH) solution, obtained by dissolving 25.77 g of $C_4H_{13}NO$ (Sigma-Aldrich, Steinheim, Germany) in 100 mL of DDW.

### Sampling

Healthy thyroid glands were collected from men who were 17–60 years old. These people died in violent accidents, such as car accidents and injuries by firearm or white weapons. All the deceased were citizens coming

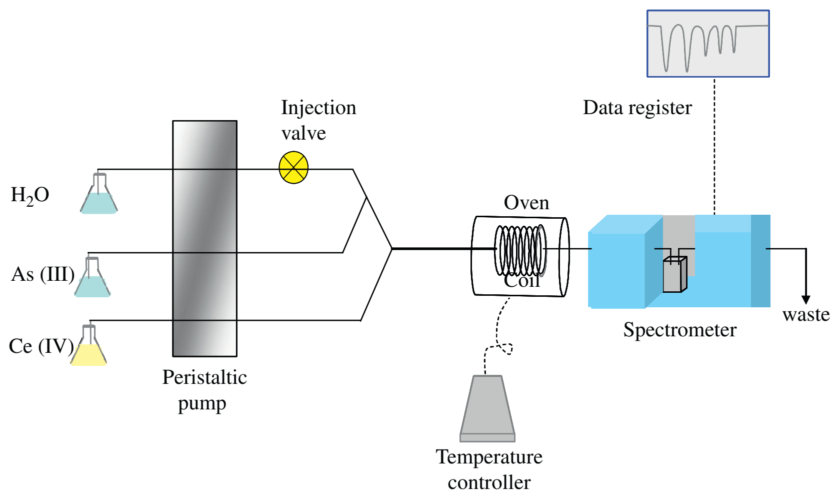


**Fig. 1.** Instrumental set-up for iodine determination using the Sandell–Kolthoff reaction.

from Caracas city, Venezuela. It is noteworthy to note that the only reason that thyroids from men were analyzed was that the accidental causes mentioned above, did not lead to woman death during the sampling period. The samples were taken with titanium instruments during autopsies at the Forensic Morgue of Bello Monte, Caracas, Venezuela. The time elapsed between death and autopsy did not exceed 24 h. By macroscopic tissue examination, the glands were considered healthy because no macroscopic anomaly (nodular masses, necrosis) was observed. Other features, such as neck volume, the presence of thyroid gland at normal location, the presence of each lateral thyroid lobe, the presence of fat, the shape and size of the lobes were also indicators of the gland healthiness. The samples were cut into two sections (left and right lobes), weighed and kept frozen at −20 °C until analysis. Iodine was determined in two replicates of each lobe and iodine concentration of the thyroid was reported as the average of lobes. Total iodine content was calculated taking into account the whole weight of the gland.

## Sample digestion

Thyroid samples were digested as follows: about 200 mg of the thyroid gland lobe was weighed accurately into a 30 mL Pyrex glass tube, then 1 mL of 25% (w/v) TMAH was added and the mix was warmed for 1 h at 90 °C in a water bath. After cooling at room temperature, the solution was diluted until 25 mL in a volumetric flask. Before the analysis, the sample solution was diluted 100-fold. This digestion procedure was optimized in a previous study [17] and no alterations (increase or loss) on iodine content were found using this methodology.

## Determination procedure

The total iodine content in the thyroid glands was determined using the flow injection spectrophotometric method based on the Sandell–Kolthoff reaction [16]. The method is based on a catalytic effect of iodine in the oxidation–reduction process between Ce (IV) and As (III). The experimental conditions are presented in Table 1. The optimal values were obtained through statistical optimization (factorial design). The following analytical figures of merits were obtained: dynamic range of 5–200 μg/L, the determination coefficient of the calibration curve was 0.9994, detection limit (LOD) ($3\sigma$) was 1.6 μg/L and the quantification limit ($10\sigma$) was 5 μg/L. It is important to state that this LOD is the lowest reported than those previously reported in clinical samples [18–20].

**Table 1.** Experimental conditions for iodine determination using Sandell–Kolthoff spectrophotometry procedure.

| Conditions | Value |
|---|---|
| Sample loop (μL) | 20 |
| Reaction coil length (cm) | 150 |
| Reaction coil temperature (°C) | 35 |
| As solution flow rate, mL/min (solution concentration: 0.008 M) | 1.2 |
| Ce solution flow rate, mL/min (solution concentration: 0.1 M) | 1.6 |
| Carrier flow rate, mL/min ($H_2O$) | 0.8 |
| Working wavelength, nm (at the maximum of $Ce^{4+}$ band) | 420 |

## Validation of the methods

The accuracy of the method was checked by analyzing the oyster tissue (NIST 1566a) certified reference material because there is no thyroid gland or similar tissue certified reference materials commercially available with certified iodine content.

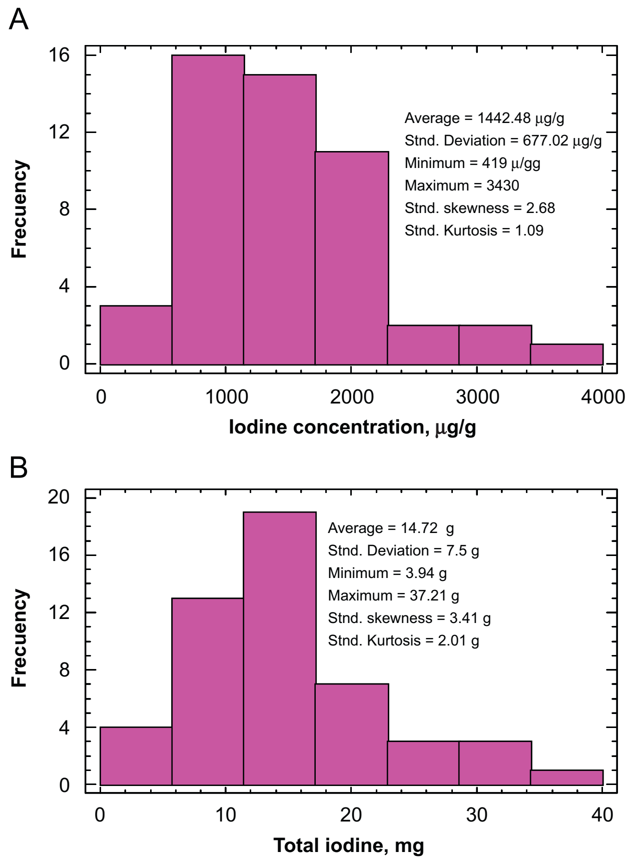


**Fig. 2.** Frequency histogram and the main descriptive statistics obtained in the analysis of 50 thyroid glands: (A) iodine concentration and (B) total iodine in the gland.

## Results and discussion

### Accuracy of the method

In order to test the accuracy of the proposed methodology, the standard reference material oyster tissue (NIST 1566a) was analyzed using the optimum conditions found in this work. The iodine mean concentration found was 4.54 ± 0.09 μg/g, which has no significant difference with the certified value of 4.46 ± 0.42 μg/g at the 95% confidence level.

### Determination of iodine in thyroid samples

The frequency histogram and the main descriptive statistics for the analysis of iodine in 50 male thyroid glands are shown in Fig. 2. The figure shows the thyroid iodine concentration (Fig. 2A) and the total iodine content in the gland (Fig. 2B). The test of goodness of fit shows, in both cases, that a normal distribution could be assumed. The median concentration obtained in the wet tissue was 1443 ± 677 μg/g (419–3430 μg/g). The mean weight of the thyroid gland was 10 ± 2 g (5.62–17.65 g) and the mean of total iodine content was 15 ± 8 mg (4–37 mg). Table 2 lists the iodine concentrations, the total iodine content and the weight of thyroid gland reported in different geographic zones in order to compare the results. Interestingly, iodine concentrations in Caracas population are higher than those values reported in other countries. These levels suggest a high intake of this element in Caracas citizens. The long-term iodize salt consumption and the frequent ingestion of seafood (the main natural dietary source of this element) might be the most important supplies. It is worthwhile to say that a law for salt iodization was implemented in Venezuela since 1968 [21]. Consequently, data from 1999 showed that 94% of the households consume adequately iodized salt with a median iodine concentration of 36.7 μg/g [22]. Furthermore, the consumption of seafood is frequent in this country due to its geographical situation. Venezuela counts with a length of coastline of 2813 km, and it is one of the most important fishing areas of the Atlantic Caribbean. The most recent data provided by the National Statistical Institute showed that the daily fish consumption per capita was about 37.10 g in 2004. This value was comparable to the red meat consumption (45.52 g) [23].

**Table 2.** Iodine concentration in thyroid gland found in some geographical regions.

| Country | Thyroid weight (range), g | Concentration ± SD (range), μg/g | Total iodine, mg | Sample number |
|---|---|---|---|---|
| Denmark [10] | Median<br>Female: 19.3<br>Male: 22.8 | Median<br>Female: 374 ± 182<br>Male: 424 ± 177 | | Females: 61<br>Males: 156 |
| England [4] | – | Median<br>1030 ± 670 (20–3120)[a] | – | 48 |
| Austria [11] | – | Median<br>640 (218–2772)[a] | | 89 |
| Russia [12] | Average<br>14.2 | Average<br>350[a] | Average<br>5.0 mg | 57 |
| Russia [13] | Average<br>14.2 ± 0.4 | Average<br>345 ± 21[b] | | |
| Russia [9] | | 1049 ± 755[a] | | 12 |
| Sweden [14] | | | Average<br>5.2 mg (0.9–20.2)[a] | 37 |
| Venezuela | Mean<br>10 ± 2 (5.62 to 17.65) | Median<br>1443 ± 677 (419–3430)[a] | Mean<br>15 ± 8 (4–37) | 50 |

[a]Wet weight.
[b]Dry tissue.

It is important to state that median urinary iodine (UI) concentration is the accepted parameter to evaluate the iodine intake. UI values have been used as epidemiologic criteria adopted by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF) and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) to classify the level of Iodine Deficiency Disorders (IDD) in a population [24]. For monitoring progress after sustainable elimination of IDD one of the criteria of the WHO, UNICEF and ICCIDD is that less than 20% of the population should have a UI < 50 μg/L. UI results in Caracas showed a median of 149 μg/L in 7–14-year-old children [25] in 1998. A survey data in school-age children in the Los Andes, Venezuela, a region characterized, in the pass, for lower iodine intake, put in evidence that 46.1% had a more than adequate iodine level intake (200–299 μg/L) and 53.8% had excessive iodine intake (equal or above 300 μg/L) with a median of 280 μg/L [26,27] in 2000–2001. These results are in accordance with the hypothesis that Caracas residents have been exposed to an excessive iodine intake.

It is also known that high iodine intake can produce iodine-induced hyperthyroidism and autoimmune thyroid diseases. For instance, in Brazil, it has been reported that the prevalence of chronic autoimmune thyroiditis increased after 5 years of excessive iodine intakes [28]. In contrast, a clinical-epidemiologic study made in Caracas from 1961 to 2004, concerning the sub-acute thyroiditis concluded that it is an infrequent disease on this region [29].

The weight of the thyroid gland in Caracas residents was lower than those reported in subjects of Denmark [10] and Russia [12,13]. In the present study, the weights represent the sum of each of the two lobes excluding the isthmus. The thyroid weight after years of influence of iodized salt has been reviewed [30]. Until the middle of the past century, a normal thyroid gland was considered to be about 20–25 g with the accepted upper normal size of 30 g, while more recent studies in iodine replete population have reported mean weights of about 10 g and an upper normal size 20 g [30].

## Statistical correlations

Fig. 3 presents the following correlations: left lobe iodine concentration with right lobe iodine concentration (Fig. 3A), iodine concentration in the gland with age (Fig. 3B) and iodine concentration of the gland with weight (Fig. 3C). No statistically significant differences in iodine concentration between the left and right thyroid lobes were found, according to a *t*-paired test (Fig. 3A; $t = 0.146$, $P > 0.05$). This result confirms the data obtained by others studies such as Zaichick and coworkers [9,12]. No correlation was either found with the age of the persons (Fig. 3B). However, a low iodine concentration with low data dispersion for people older than 40 years old was observed. No correlation was found between the gland's weight and the intrathyroidal iodine concentration (Fig. 3C).

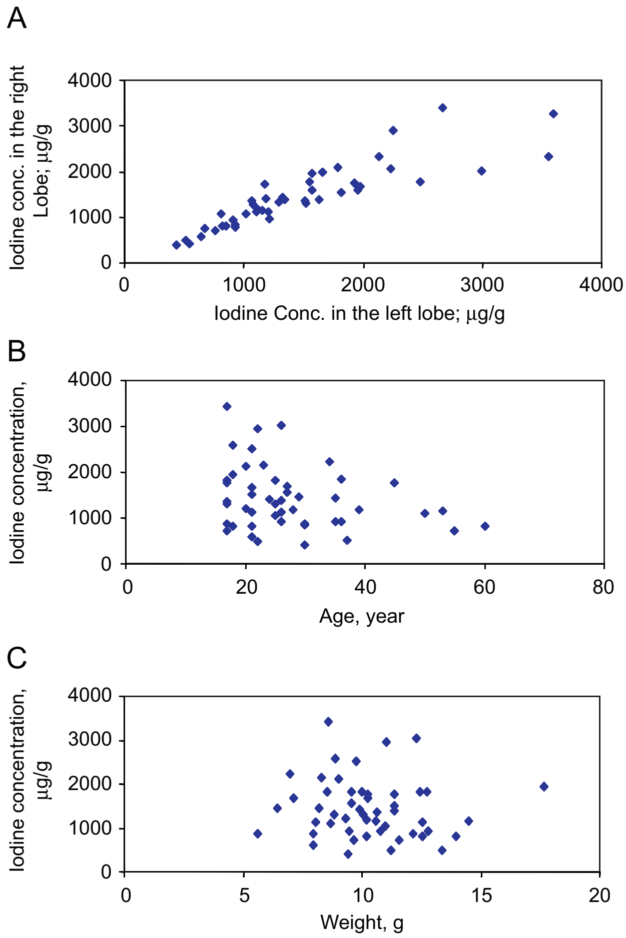


**Fig. 3.** Correlation of intrathyroidal iodine concentration as function of the (A) right and left lobes, (B) age and (C) total weight of the gland.

## Conclusions

The median iodine concentration measured in a group of thyroid gland tissues from healthy bodies was 1443 ± 677 μg/g with a median of total content in the gland of 15 ± 8 mg. This intrathyroidal iodine content is higher than other worldwide values maybe due to high intake of this element from iodinated salt and the frequent consumption of seafood of the Caracas population. No correlation between iodine concentration in the thyroid and the size or weight of the glands was detected. The thyroid weight of Caracas population agrees with what is seen in populations with high iodine intake levels.

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## References

[1] Markou K, Georgopoulus N, Kyriazopoulou V, Vagenakys A. Iodine-induced hypothyroidism. Thyroid 2001; 11:501–10.
[2] Roti E, Degli E. Iodine excess and hyperthyroidism. Thyroid 2001;11:493–500.
[3] Hoption S. Hypothesis: dietary iodine intake in the etiology of cardiovascular disease. J Am Coll Nutr 2006; 25:1–11.
[4] Tadros TG, Maisey MN, Ng Tang Fui SC, Turner P. The iodine concentration in benign and malignant thyroid nodules measured by X-ray fluorescence. Br J Radiol 1981;54:626–9.
[5] Zaichick V, Matveenko EG, Vtiurin BM, Medvedec VS. Intrathyroid iodine in the diagnosis of thyroid cancer. Vopr Onkol 1982;28:18–24.
[6] Malenchenko A F, Demidchik E P, Tadeush V N. Content and distribution of iodine, chlorine and bromine in normal and pathologically changed thyroid gland tissue. Med Radiol (Mosk) 1984;29:19–22.
[7] Zaichick V, Tsyb A, Vtyurin B. Trace elements and thyroid cancer. Analyst 1995;120:817–21.
[8] Bellisola G, Brätter P, Cinque C, Francia G, Galassini S, Gawlik D, et al. The TSH-dependent variation of the essential elements iodine, selenium and zinc within human thyroid tissues. J Trace Elem Med Biol 1998;12:177–82.
[9] Zaichick V, Zaichick SV. Energy-dispersive X-ray fluorescence analysis of iodine in thyroid punctures biopsy specimens. J Trace Microprobe Techn 1999;17:219–32.
[10] Agerbaek H. Weight and iodine content of the thyroid gland in Jutland, Denmark. Acta Med Scand 1974; 196:505.
[11] Tiran B, Karpf E, Tiran A, Lax S, Langsteger W, Eber O, et al. Iodine content of thyroid tissue in the Styrian population. Acta Med Austriaca 1993;20:6–8.
[12] Zaichick V, Choporov Y. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J Radioanal Nucl Chem 1996;20:153–61.
[13] Zaichick V, Zaichick S. Normal human intrathyroidal iodine. Sci Total Environ 1997;206:39–56.
[14] Milakovic M, Berg G, Eggertsen R, Nystrom E, Olsson A, Larsson A, et al. Determination of intrathyroidal iodine by X-ray fluorescence analysis in 60-to 65-year olds living in an iodine-sufficient area. J Intern Med 2006; 260:69–75.
[15] Murillo M, Carrión N, Quintana M, Sanabria G, Ríos M, Duarte L, et al. Determination of selenium and iodine in human thyroids. J Trace Elem Med Biol 2005;19:23–7.
[16] Sandell E, Kolthoff I. Chronometric catalytic method for the determination of microquantities of iodine. J Anal Chem Soc 1934;56:1426–30.
[17] Zabala J, Carrión N, Quintana M, Murillo M, Chirinos J, Seijas N. Fifth Internacional Symposium on Trace Elements in Human: New Perpectives, Athens, Greece, October 13–15, 2005.
[18] Tsuda K, Namba H, Nombra T, Yokoyama N, Yamashita S. Automated measurement of urinary iodine with use of ultraviolet irradiation. Clin Chem 1995;41: 581–5.
[19] Yaping Z, Dongxing Y, JIxiang C, Tianshiu L, Huiqin C. Spectrophotometric determination of urinary iodine by flow-injection analysis with on-line catalytic digestion. Clin Chem 1996;42:2021–7.
[20] Nacapricha D, Muangkaew S, Ratanawimarnwrong N, Shiowatana J, Grudpan K. Continuous and stopped flow injection for catalytic determination of total iodine in urine. Analyst 2001;26:121–6.
[21] Pretell E, Delange F, Hostalek U, Corigliano S, Barreda L, Higa AM, et al. Iodine nutrition improves in Latin America. Thyroid 2004;14(8):590–9.
[22] ICCIDD. The IDD program in Venezuela. IDD Newlett 1999;15(4):1.
[23] 〈http://www.ine.gov.ve/consumo/seleccionconsumo.asp〉.
[24] WHO, UNICEF and ICCIDD. Assessment of the Iodine Deficiency Disorders and monitoring their elimination. Geneva: WHO Publication; 2001 WHO/NHD/01.1:1-107.
[25] FAO. Perfiles nutricionales por Países. Venezuela: FAO; 2000.
[26] Caballero L, Cárdenas L, Omaña M, Avendaño T. Excreción urinaria de yodo (yoduria) en escolares de la región andina de Venezuela durante el periodo 2003–2005. Revista electrónica de portales medicos.com. Publ 2007.
[27] De Benoist B, Andersson M, Egli I, Takkouche B, Allen H. Iodine status wordwide: WHO global datebase on iodine deficiency. WHO Publ, 2004. p. 1–11.
[28] Camargo R, Knobel M, Medeiros-Neto G. Iodine nutrition: more is better? Arq Bras Endocrinol Metab 2007;51(4):639–40.
[29] Marsigilia I. Estudio clínico y epidemiológico de la tiroiditis subaguda (tiroiditis granulomatosa de de Quervain). Gac Méd Caracas 2006;114(4):273–90.
[30] Langer P. Discussion about the limit between normal thyroid and goiter: minireview. Endocr Regulat 1999;33: 39–45.