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Ciências da Saúde

Iodine Deficiency and Thyroid Nodular Pathology- Epidemiological and Cancer Characteristics in Different Populations

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I dedicate this Thesis to my family, including my ever present and caring mother, my devoted father and the doyen of the family, my late grandfather Manuel Pereira Santos, on whose birthday I was born one late July.

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To God, to whom I owe life and everything that I have achieved in its many aspects. For being alive against all statistical odds, and for enabling me to achieve this research project that hopefully will benefit the people of Portugal, and all the many countries throughout the world where iodine deficiency still prevails.

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Resumo Alargado

O iodo é o componente essencial das hormonas tiroideias triiodotironina (T3) e tiroxina (T4). Estas hormonas são fundamentais para o metabolismo celular em todas as células do corpo. Na fase embriológica da vida as hormonas tiroideias são ainda fundamentais para o desenvolvimento normal de todos os órgãos sendo particularmente importantes para o desenvolvimento cerebral.

Globalmente o défice de iodo afecta em excesso de 2 biliões de pessoas de pelo menos 130 países, entre as quais se estima que estão 266 milhões de crianças em idade escolar.

O défice de iodo está associado com problemas de desenvolvimento cognitivo, comprovados défices nas funções neuropsicointelectuais, comprovados problemas reprodutivos, no desenvolvimento de bócio e de patologia nodular da tiroide bem como um aumento na incidência do cancro da tiroide. Com o défice de iodo ocorre também uma alteração do perfil da patologia do cancro da tiroide com um aumento das incidências relativas dos cancros tiroideus mais agressivos como o cancro tiroideu com predominância do carcinoma folicular da tiroide e um aumento na incidência dos carcinomas anaplásicos. Isto ao contrário dos tumores predominantes em áreas sem défice de iodo, os carcinomas papilares habitualmente de comportamento indolente.

Em 1990 numa cimeira nas Nações Unidas em Nova Iorque intitulada “World Summit for Children 1990” que contou com a presença de 71 Chefes de Estado foi assinada por todas as Nações presentes uma série de declarações de intenções para o bem de todas as crianças de todo o mundo entre as quais estava a da eliminação do défice de iodo até ao ano 2000. Para completar a universalidade destas moções elas foram subsequentemente ainda assinadas por mais 88 Governos que não tinham assinado na altura.

Passaram-se mais de 25 anos desde essa conferência e a prevalência do défice de iodo continua a um nível superior ao dos 2 mil milhões de pessoas. Isto revela medidas ineficazes tomadas (ou não) por governos que não têm em conta o interesse do Povo.

Países como a República Popular da China e a África do Sul são exemplos em que medidas políticas eficazes foram adoptadas para corrigir este problema tendo na China o Congresso do Povo decretado que a iodização do sal era no interesse nacional e no interesse do Povo e como tal os (pequenos) custos da iodização do sal passaram a ser pagos pelo Estado. Para que essa correção deste problema seja possível é necessário que haja a disponibilidade de sal iodado com teor certificado por todo o País e que o mesmo seja a um preço acessível. Estas medidas foram implementadas nos Países mencionados (China e África do Sul), previamente documentados como

deficitários de iodo e que após a introdução das medidas corretivas necessárias foram documentados como tendo conseguido eliminar o problema do défice de iodo.

Dados epidemiológicos sobre a nutrição de iodo a nível Nacional e Regional são essenciais para o desenvolvimento de estratégias eficazes para a eliminação do problema do défice de iodo. Portugal é reportado nos artigos de revisão sobre o estado da nutrição de iodo no mundo como um dos países de onde não existem dados nacionais populacionais sobre a nutrição de iodo.

A nutrição de iodo é hoje em dia avaliada através dos valores da excreção urinária de iodo medidas através do valor da mediana da concentração urinária do iodo (urinary iodine concentration – UIC). Um valor de UIC inferior a 100 µg/L é classificado como deficitário de iodo. Outros estudos referem parâmetros alternativos de prevalência de bócio e de patologia nodular da tiroide e o seu tamanho. O perfil de anatomia patológica das tiroidectomias é também tido como informação complementar com os carcinomas papilares a predominarem em áreas não deficitárias de iodo sobre os carcinomas foliculares com rácios papilares / foliculares (P:F) de 3.4:1 a 6.0:1 reportados nos Estados Unidos da América em áreas que não são deficitárias de iodo. Em contraste em áreas deficitárias de iodo o perfil é alterado observando-se um aumento relativo dos cancros tiroideus mais agressivos com uma maior incidência relativa de carcinomas foliculares com alteração do rácio P:F para cerca de 1 ou <1. Nas regiões onde o défice de iodo é prevalente há ainda um aumento na incidência dos tumores mais agressivos, os carcinomas anaplásticos da tiroide.

Dados relativos á nutrição de iodo a nível Nacional sobre Portugal só surgiram em 2010 através de uma publicação internacional sobre o estado da nutrição de iodo na população específica das grávidas. Esse estudo revelou défice de iodo em todas as regiões do País com um valor mediano global, nacional de UIC de 82.5 µg/L. As duas áreas mais deficitárias de iodo foram a ilha de S. Miguel nos Açores, com um valor mediano de UIC de 50.0 µg/L, seguido o da região da Beira Interior com um valor de UIC de 67.6 µg/L.

No estudo presentemente realizado respeitante á excreção urinária de iodo, avaliado em 214 voluntários da população geral (131 do sexo feminino e 83 do sexo masculino) com idades entre os 8 e os 97 anos residentes na região da Beira Interior (BI) o valor mediano do UIC foi de 62.6 µg/L. Mais de um terço da amostragem (n=76/214) tinha um valor inferior ao limiar classificado como muito baixo de 50 µg/L e mais de 92% das amostragens (197/214) um valor inferior a 100 µg/L.

O resultado obtido para a mediana de UIC da população geral da região da BI de **62.6 µg/L** é relativamente semelhante ao obtido para a população específica de grávidas respeitantes á região da BI, no único estudo Nacional onde os valores da UIC foram determinados: UIC de **67.6 µg/L**. Tendo em conta os hábitos da cultura Portuguesa em que a maior parte (definido como > 50%) das pessoas tende a comer comida preparada em casa, muitas vezes comum ao resto da família, dada a falta de dados Nacionais da população geral, respeitantes á nutrição de iodo, pode presentemente assumir-se que os dados Nacionais publicados sobre a avaliação da população de grávidas provavelmente serão extrapoláveis para a situação na população geral. Essa inferência de dados prováveis em nada altera a importância e prioridade de ser feito um estudo Nacional documentando a nutrição de iodo na população geral.

Como evidência complementar sobre a nutrição de iodo da população da BI foi avaliado o perfil da patologia nodular da tiroide na população da BI, através da anatomia patológica da tiroide da população da Beira Interior reflectida através dos Instituto Português de Oncologia de Coimbra, Porto e Lisboa, respeitantes a doentes com a área de residência oriunda da região da BI, bem como dos Hospitais da Covilhã e do Fundão, num período de 6 anos de Janeiro de 2002 a Dezembro de 2007.

Este perfil foi comparado com o perfil de anatomia patológica da cirurgia da tiroide feito no período de 5 anos, Janeiro de 1984 a Dezembro de 1988, na região de Joanesburgo, na África do Sul. Este período corresponde a uma altura anterior á introdução da iodização mandatória de todo o sal para consumo humano, feita em legislação introduzida em 1995. Nessa altura de avaliação, a determinação de UIC ainda não se efetuava.

Apesar dos períodos diferentes da colecção dos dados eles correspondem a períodos em que havia défice de iodo.

Os perfis de anatomia patológica foram avaliados e comparados estatisticamente analisando as frequências relativas das neoplasias e os dados calculados através do qui-quadrado (G* Power) e do intervalo de confiança a 95% e eram sobreponíveis como é reflectido na Tabela 1.

Na África do Sul o défice de iodo está documentado como tendo sido eliminado depois da introdução da iodização obrigatória do Sal. A introdução de uma medida semelhante aliada a campanhas de informação popular acerca das consequências do défice de iodo seriam medidas que teriam a possibilidade de contribuir para a eliminação do défice de iodo em Portugal. Dado que Portugal tem produção comercial de sal essas medidas seriam facilmente implementáveis. Promover a nutrição á base dos produtos do mar, que são naturalmente ricos em iodo, seria uma medida adicional recomendável.

Consciencialização da importância eliminação do problema do défice de iodo deverá contribuir para a realização do primeiro estudo nacional que documente a nutrição de iodo na população geral de Portugal.

Palavras-chave

Tiroide; Défice de iodo; Nutrção de iodo; Bócio; Patologia nodular da tiroide; Carcinoma da tiroide; Carcinoma papilar da tiroide; Carcinoma folicular da tiroide; Carcinoma anaplástico da tiroide; Concentração urinária de iodo; Sal iodado; Nutrição de produtos marinhos;

Abstract / Thesis Overview

Iodine is the essential component of the thyroid hormones T3 and T4, which regulate metabolic processes in most cells and play an important role in the early growth and development of most organs, particularly the brain. Globally iodine deficiency (ID) is the most common preventable cause of brain damage, with more than 2 billion people from 130 countries at risk, (241 million children of school age). It is especially prevalent worldwide in inland continental or mountainous regions, and may be independent of sea proximity. Consequences of ID include variable degrees of intellectual impairment, with demonstrable neuropsychointellectual deficits, compromised reproductive potential, development of goitre, thyroid nodular pathology and an increase in the incidence of thyroid cancer.

There is still no general population data on iodine nutrition (IN) from Portugal. This study aims at evaluating the IN of the general population of the inland region of Beira Interior (BI) in Portugal and to compare their parameters with the available IN parameters for the equally high altitude region of Johannesburg (JHB), South Africa prior to legislation enforcing the iodisation of all manufactured food grade salt in 1995.

In this thesis after an introductory overview of the historical aspects relating to iodine, ID and the mechanisms for goitre, hyperplasia, nodular and cancer development, the relationship between the environment and ID is analysed together the methods for evaluation of IN. Thyroid nodules, their benign and malignant characteristics on different investigation modalities are discussed, after a preliminary overview of the thyroid anatomy, histology and physiology.

The IN of the population of BI was evaluated through combined parameters of the urinary iodine concentration (UIC) of a general population sample of 214 volunteers and the thyroid histology pattern of patients from the study area of BI operated over the 6 year period January 2002 to December 2007. This was compared to the available nutritional parameter of IN in the JHB region before the mandatory salt iodization, the thyroid histology pattern over the 5 years: January 1984 to December 1988.

The median UIC from the population of BI revealed significant ID. The thyroid histology patterns were characteristic of ID in both regions with a statistically significant overlap of the results.

The adoption of legislation requiring all food grade salt to have a minimum amount of iodine and the promotion of sea based nutrition in a well informed public that could be pro-active in the elimination of ID would be important in achieving this goal.

Keywords

Thyroid; Iodine deficiency; iodine nutrition; goitre; thyroid cancer; papillary thyroid cancer; follicular thyroid cancer; anaplastic thyroid cancer; urinary iodine concentration; iodized salt; seafood nutrition.

Table of Contents

Dedication iii

Acknowledgements v

Resumo Alargado vii

Palavras Chave x

Abstract / Thesis Overview xi

Keywords xii

Table of Contents xiii

List of Figures xvi

List of Tables xviii

Abbreviations xx

Chapter 1 Introduction 1

1 Aim of study 1;7

1.1 Historical developments relating to iodine and the thyroid 2

1.2 Iodine deficiency 3

1.3 Iodine deficiency and mechanisms for goitre, thyroid nodular and cancer development 4

Chapter 2 The environment and iodine deficiency disorders 8

2.1 Water reservoirs 8

2.2 Sources of iodine intake – nutrition 9

2.3 Evaluation of Iodine Nutrition 9

2.3.1 Goitre 10

2.3.2 Urinary Iodine excretion 10

2.3.3 Histology findings 10

2.3.4 Thyroglobulin 12

Chapter 3 Thyroid Nodules – Methods of Diagnosis: Benign and Malignant Characteristics. Iodine Deficiency and Thyroid Cancer 13

3.1 Thyroid Anatomy 13
3.2 Physiology of the Thyroid gland 15
3.3 Clinical Evaluation 16
3.4 Ultrasonography 17
3.4.1 Thyroid ultrasound elastography (USE) 20
3.5 Radionuclide Imaging 20
3.6 Cytology 20
3.7 Histology 21

Chapter 4 Material and Methods 23

4.1 Material 23
4.1.1 Study design 23
4.1.2 Cytology 24
4.1.3 Histology - Diagnosis evaluation by Histology 24
4.1.4 Urinary Iodine Excretion – Population sample and measurements 25
4.1.5 Study Population 26
4.2 Methods 26
4.2.1 Cytology 26
4.2.1.1 Fine Needle Aspiration Biopsy 26
4.2.1.2 Fine Needle Nonaspiration (Capillary) Cytology 28
4.3 Statistical analysis 34

Chapter 5 Results 35

5.1 Cytology and Histology data from the Region of Beira Interior 35
5.2 Histology results - Regions of Beira Interior and Johannesburg 37
5.3 Combined evaluation of Iodine Nutrition - Beira Interior 40

Chapter 6 Discussion 42

Chapter 7 Publications in support of this Thesis: Published Articles and Abstracts 47

Chapter 8 Conclusions and Future Perspectives 83

References 85

Appendix 93

List of Figures

Figure 1. Anterior view of the thyroid gland displaying its relations to the adjacent structures. Adapted with permission from McHenry CR Thyroidectomy and Parathyroidectomy; Chapter 3; 21-36 https://clinicalgate.com/thyroidectomy-and-parathyroidectomy-2/

Figure 2. Inset of thyroid gland histology displaying colloid filled thyroid follicles. Each follicle is surrounded by a delicate layer of loose connective tissue. A capillary is also displayed. Adapted w/ permission from Histology-Endocrine http://www.ouhsc.edu/histology/Glass%20slides/42_03.jpg Copyright © 2016 The Board of Regents of the University of Oklahoma.

Figure 3. Physiological principles of thyroid and the pituitary – hypothalamic axis. Adapted with permission from Browse´s Introduction to the Symptoms and Signs of Surgical Disease, Fourth Edition 2005.

Figure 4. Clinical findings related to risk of carcinoma in a thyroid nodule. Adapted with permission from: "Werner & Ingbar´s The Thyroid A Fundamental and Clinical Text, Tenth Edition, 2013; Chapter 49, Clinical evaluation and management of thyroid nodules.

Figure 5. Diagnostic Index for Individual Sonographic Criteria of Malignant Thyroid Nodules Adapted with permission from Kim E-K, et al. Am J Roentgenol 2002; 178:687-691.

Figure 6. Sonographic Findings in 155 Maignant or Benign Thyroid Nodules. Adapted with permission from Kim E-K, et al. Am J Roentgenol 2002; 178:687-691

Figure 7. Ultrasound features associated with thyroid cancer. Adapted with permission from Frates MC, et al. Radiology 2005;237:794-800.

Figure 8. (a.) Sagittal US image of a nodule (arrow-heads) containing multiple fine echogenicities (arrow) with no comet-tail artefact. These are highly suggestive of malignancy. FNA and surgery confirmed a papillary carcinoma. **(b.)** Transverse US image of nodule (arrowheads) containing cystic areas with punctate echogenicities and comet-tail artefact (arrow) consistent with colloid crystals in a benign nodule. Adapted from: Frates MC, et al. Management of Thyroid Nodules at US: Society of Radiologists in Ultrasound Consensus Conference Statement. Adapted with permission from Radiology 2005; 237:794-800.

Figure 9. FNAB performed with a mechanical syringe holder with the nodule isolated and stabilized. From: Santos JEC 1995. The role of Fine Needle Cytology in Thyroid Disease. Thesis (Master of Medicine) University of Witwatersrand, Johannesburg, South Africa.

Figure 10. Demonstration of Fine Needle Nonaspiration Cytology. The needle is gripped like a pencil, moving it in and out and in different directions within the nodule being sampled. From: Santos JEC, Leiman G. Nonaspiration Fine Needle Cytology- Application of a New Technique to Nodular Thyroid Disease. Acta Cytol 1988; 32:353-356, S. Karger AG, Basel.

Figure 11. The principle of capillary tension. The ascent of fluid into a narrow channel is governed by the formula $h = 2\gamma/pgr$, where h is the height attained, γ is the fluid surface tension, p is the density of that fluid, g is the gravity and r is the radius of the tube.
From: Santos JEC, Leiman G. Nonaspiration Fine Needle Cytology- Application of a New Technique to Nodular Thyroid Disease. Acta Cytol 1988; 32:353-356, S. Karger AG, Basel.

Figure 12. FNAB performed with the needle being moved in different directions within the nodule to be sampled whilst aspiration is applied. From: Santos JEC 1995. The role of fine needle

cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

Figure 13. The aspirated material is transferred onto the glass slide. From: Santos JEC 1995. The role of fine needle cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

Figure 14. Preparation of the smear which is then either immediately fixed with an alcohol based spray, for subsequent staining according to the method of Papanicolau or air dried , the slide being labeled as such, and subsequently stained by the May-Grunwald-Giemsa method. From: Santos JEC 1995 The role of fine needle cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

Figure 15. The relative frequencies of the different types of thyroid nodular pathology encountered in the population of the iodine deficient area of Beira Interior in Portugal for the 6 year period January 2002 to December 2007.

Fig. 16. Histological diagnosis: Thyroid malignancies – Beira Interior: 6 year period (January 2002-December 2007). The types of follicular cancer are discriminated.

Fig 17. Histological diagnosis: Thyroid malignancies – Johannesburg: 5 year period (January 1984– December 1988). The types of follicular cancer are discriminated

Fig. 18: Urinary Iodine Concentration (UIC) in population sample of 214 volunteers (131♀; 83♂) with ages ranging 8 to 97 years (median 52.0 years).

List of Tables

Table 1 - Frequency of different pathology types in the two study areas.

Abbreviations

ATC - anaplastic thyroid cancer;
BH - Baragwanath Hospital;
BI - Beira Interior;
c.c. - cubic centimetres;
CI - confidence interval;
EG - endemic goitre
H&E - hematoxilin and eosine;
FNBC - fine needle biopsy cytology
FTC – follicular thyroid cancer;
IC – iodine content;
ICCIDD - International Council for the Control of Iodine Deficiency Disorders
ID - iodine deficiency;
IDD - iodine deficiency disorders;
IN - iodine nutrition;
IR - incidence rates
JHB - Johannesburg;
mL - millilitre;
MGG - May-Grunwald-Giemsa stain;
P:F – Papillary to follicular;
PTC - papillary thyroid cancer;
SA - South Africa
T3 – tri-iodothyronine
T4 – thyroxine
TSH - thyroid stimulating hormone;
TC – thyroid cancer;
Tg - thyroglobulin;
UIC - urinary iodine concentration;
UK – United Kingdom;
UNICEF - United Nations Nations Children's Fund
US - ultrasound
USA – United States of America;
USE - ultrasound elastography;
WHO - World Health Organization

Chapter 1

Introduction

Iodine is the essential component of the thyroid hormones tri-iodothyronine (T3) and tetra-iodothyronine or thyroxine (T4) [1], which regulate metabolic processes in most cells and play an important role in the early growth and development of most organs, particularly the brain [2].

Globally iodine deficiency (ID) is the most common preventable cause of brain damage, with more than 2 billion people from 130 countries at risk [3], including an estimated 241 million children of school age [4]. Iodine deficiency is especially prevalent worldwide in inland continental or mountainous regions (1), in areas beyond high mountain ranges [5], and it may be independent of sea proximity.

Consequences of ID include variable degrees of intellectual impairment, with demonstrable neuropsychointelectual deficits [6,7], compromised reproductive potential [8], development of goitre, thyroid nodular pathology [1] and an increase in the incidence of thyroid cancer (TC) [9,10].

In areas where iodine sufficiency exists papillary thyroid cancer (PTC) is the predominant form of thyroid cancer [11,12], with papillary to follicular (P:F) ratios of 3.4-6.0:1 reported from the USA [12]. By contrast in iodine deficient areas follicular thyroid cancer (FTC) tends to predominate [11,12,13] with a consequent inversion in the usual frequency ratio of papillary to follicular (P:F) thyroid cancer being observed. A relative increase in the incidence of anaplastic thyroid carcinomas is also observed in ID areas [9,12,13]. The occurrence of higher percentages of the more aggressive subtypes of TC (follicular thyroid cancer (FTC) and anaplastic thyroid cancer (ATC)) is also shown in ID areas [9,10]. This was recently confirmed in a comprehensive review on iodine intake and risk factors for thyroid cancer [14].

The aim of the present study is to contribute towards the elimination of iodine deficiency in Portugal. Towards this aim the iodine nutrition (IN) of the general population of the inland region of Beira Interior (BI) in Portugal is evaluated for the first time and compared with data from the area of Johannesburg in South Africa, during a period when ID prevailed. To confirm the existence of ID in BI, apart from the thyroid histology profile analysis, the UIC of a general population sample is evaluated. As a contributing factor towards ID the iodine content of the largest surface water reservoir of BI is also evaluated and documented for the first time.

It is hoped that this regional study from BI will help spearhead a much needed National population study on IN. This data is much needed to enable correct documentation of the extent of the problem and the planning of corrective strategies.

1.1 Historical developments relating to iodine and the thyroid

Historical references relating to what we now know as the thyroid gland arise early in medical history, in Chinese Medical writings.

In 1600 BC the Chinese were known to use burnt sea sponge and seaweed for the treatment of goitres, (enlargement of the thyroid) [15]. The word goitre is derived from the Latin word: "guttur" which means throat.

The use of burnt sea sponge and seaweed was based on chance observation adjusted by empirical methods of prescribing, without knowledge of iodine or iodine deficiency as such. Chinese ancient Medicine advised the patient to take these remedies at least twice a year, in the spring and in autumn [15].

Without making reference to either the origin, or the rationale of their treatments, quite independently from each other, many years later and centuries apart, renowned physicians like Hippocrates and Galen also recommended similar remedies [15].

In the early 20th century Marine and Kimball [16] applied a similar principle, administering sodium iodide to schoolgirls twice yearly. They claimed that the results obtained from a two and a half years' observations demonstrated both its preventative and curative effects with regard to the development of enlargement of the thyroid.

Although empirical observation in ancient times had noted the beneficial effects of taking burnt sea sponge and seaweed in reducing goitre size it was only in 1656 that Thomas Wharton, a London physician, first described and named a ductless gland in the neck which he called: the thyroid gland - the shield-shaped gland [17]. This preceded the discovery of iodine by more than one hundred and fifty years [17]. At that time there was no knowledge as to the function of this gland. In his original description, Wharton merely referred to its morphologic aspects, mentioning that: "it contributed to the beauty of the neck, filling-up the vacant spaces around the larynx, making its protuberant parts smooth, particularly in females, to whom for this reason a larger gland had been assigned, rendering their necks more even and more beautiful" [17]. This comment might be a reflection of the observation that thyroid hypertrophy was more common in the female.

Iodine was discovered in late 1811 by the French chemist Bernard Courtois [15]. He noticed it as an intense violet coloured vapour arising from seaweed ash after adding, fortuitously, a stronger than usual amount of sulphuric acid [15], whilst manufacturing the essential component for gunpowder: potassium nitrate (referred to, at the time, as saltpeper). This was then critical for Napoleon's army.

Subsequently, Courtois noticed that, in the metal vats used in this preparation, a deposit of “lustrous metal like crystals“ formed. These were scraped off, examined and analysed by Courtois [17].

After several months of investigations of the more obvious chemical properties and reactions of this new substance, Courtois was forced to abandon his research through lack of money, unfortunately without ever managing to present or publish his findings and his results [15].

These lustrous metal like crystals that had been scraped off from the metal vats used in the preparation of potassium nitrate from seaweed ash were given, amongst others, to Gay-Lussac who subsequently identified their composition as being a new element [15]. He named it “iode”, from the Greek word for violet, “ioeides” [17]. This name was subsequently anglicised to “iodin” by the eminent English chemist Sir Humphry Davy who had also been given some of these crystals, before they had been identified as a new element [17].

The first publication describing this new substance appeared in 1813 in “Annales de Chimie” written by Gay-Lussac, (one of the distinguished chemists that Courtois had given these cristals to), by way of an anonymous paper [18,19]. In this publication he gave full credit to Courtois for the discovery of “iodin” [19]. “Iodin” later, in the 1930´s, became iodine [18].

Iodine is the essential component of the hormones produced by the thyroid gland [1,20]. It was detected in high concentrations for the first time in the thyroid gland by E. Baumann in 1895 [21].

The iodine containing hormone of the thyroid gland thyroxine was first isolated in crystalline form by E. C. Kendall at the Mayo Foundation in Rochester, U.S.A. who gave it its name between the years of 1913 and 1919 [17] The exact chemical constitution of iodine was subsequently determined by Sir Charles Harrington who demonstrated that the molecule of thyroxine contains four atoms of iodine [17]. This finding allowed the means for its artificial synthesis.

1.2 Iodine deficiency

The prevalence of iodine deficiency disorders (IDD) is related to the level of iodine ingestion that tends to occur in particular areas [22].This might be associated with the local conditions in existence in the area, which might predispose to an insufficient iodine intake. Iodine deficiency and the resultant IDD are still recently reported occurring in several European countries [22,23]

Iodine Deficiency (ID) tends to occur predominantly in certain geographical areas or regions. In many cases ID problems are related to the bioavailability of iodine in soils [5].The iodine content of plants is generally low and it has been demonstrated that strict vegetarian diets tend to result in low iodine intake[5], which can lead to iodine deficiency.

Iodine Deficiency soils are more common in inland central continental regions, mountainous areas [1], beyond high mountain ranges. Nevertheless ID is not just related to being away from the coastline as it also occurs in coastal regions [1], in islands [24] and even in small islands, like the island of Pemba, (the smallest of the Zanzibar islands of Tanzania) [24]. Amongst others it is also documented in the island of East Timor in the Pacific Ocean [25], previously a Portuguese Colony.

The common denominator to most of these areas of ID tends to be poverty and social disadvantage, as well as lack of social and economic development, although this is not always the case [25]. Above all, one is lead to feel that the dominant factor in all these areas is mostly lack of education [24] and of awareness as to the mechanisms that are associated with ID and what its consequences are.

1.3 Iodine deficiency and mechanisms for goitre, thyroid nodular and cancer development

Low iodine intake leads to an adaptive process that results in a sustained increased secretion of thyroid stimulating hormone (TSH), in an effort to maximise uptake of the available iodine [1]. TSH also has a direct trophic effect on the thyroid gland, stimulating thyroid hypertrophy and hyperplasia. Initially goitres are characterised by diffuse, homogeneous enlargement that over time progresses onto nodular development [1]. Persistent low iodine intake can be the factor for the morphologic alterations observed in endemic goitre [26], with an initial diffuse hyperplasia.

In patients with endemic goitre the morphologic changes observed consist of nodular enlargement of the thyroid gland resulting from marked microscopic and macroscopic heterogeneity [27].The understanding of the basis for the development of this heterogeneity stems from two basic, yet fundamental findings: that the follicular cells of a normal thyroid are not identical amongst themselves and that daughter follicles which are produced during a period of goitrogenesis, arise from follicular cells which are endowed with an inherited propensity to replicate at an higher than average rate [27].These resulting cells might also have the capacity to fine tune the function of the thyroid gland in response to changing demands. These two new findings allowed for the understanding of the processes of diversity in the progeny of the growing cell population of the thyroid.

The thyroid, like other organs [28], naturally contains a small fraction of cells with high inborn replication power. This property is a stable cell trait that is passed onto daughter cells. This process is accelerated by chronic stimulation of the gland, leading, in time, to the development of focal hyperplasia and adenoma formation [27]. This may be relevant not only to the pathogenesis of benign nodules and adenomas, but it might ultimately also be linked to the occurrence of autonomous growth and even to malignant transformation [27].

Thyroid nodules are common in clinical practice. Their reported prevalence depends on the characteristics of the population being studied and on the diagnostic and screening methods used. It increases with age and is reported as being higher in women, in people with iodine deficiency and in individuals that have been exposed to head and neck radiation [29,30]. Palpation is the least sensitive of all diagnostic methods in the detection of thyroid nodules and collated reports from numerous studies suggest a prevalence of 2-6% with an average value of approximately 4% [29], around 6.4% in females and 1.5% in males [30].

The described prevalence of thyroid nodules on ultrasound examination ranges from 19 to 35% [29], and as high as 67% if high-resolution ultrasound is used [29,31]. It is, however, important to be aware that these incidentally found nodules do not appear to have clear clinical significance, hence their designation as incidentalomas. Since the description and naming of incidentalomas in 1994 [31], several sonographic criteria have been established that aid in determining which nodules should be considered for further cytological investigation. These are: the finding of microcalcifications suggestive of malignancy (tiny,punctate hyperechoic foci), irregular or microlobulated margins, marked hypogenicity, as well as the nodule shape/dimension ratio: in that the nodule being taller than wider indicates a higher likelihood of it being malignant [32].

Subsequently the Society of Radiologists in Ultrasound (USA) issued their consensus statement Guidelines [33] on which nodules should be subjected to ultrasound (US) guided FNAB and which should not. The American Association of Clinical Endocrinologists has also produced their Guidelines jointly with the Italian Association of Endocrinologists and the European Thyroid Association [34].

These three sets of Guidelines were analysed in a study that evaluated the results of a total of 1,398 nodules through fine needle biopsy cytology (FNBC) or Surgery for the sensitivity, specificity and accuracy of the above mentioned Guidelines criteria [35].

All these criteria and the recently developed imaging technique of thyroid ultrasound elastography (USE) will be discussed in further detail in Chapter 3, Section 3.2.

Autopsy data is regarded as the gold standard for determining the true prevalence of thyroid nodules [29]. Several autopsy studies have been performed in non-endemic and endemic goitre areas and the prevalence of thyroid nodules in these studies displayed a wide range of 82-646 per 1000 autopsies [36]. The variability of these reported rates is thought to be attributable to different levels of meticulousness on the part of the examining pathologists.

In spite of thyroid nodules being common in clinical practice thyroid cancer is relatively uncommon, being more frequent in women. It is, nevertheless the most common endocrine malignancy with documented increasing incidence rates in most countries [14].

Iodine deficiency is known to have an influence in TC with ID having been demonstrated to have induced Thyroid malignancy in rats [37], and in mice [38]. A statistically significant increased prevalence of thyroid cancers was documented in an iodine deficient area in Sicily, Italy, when compared to an adjacent area where there was no ID [10]. The frequency of thyroid cancers was also shown to be different with follicular and anaplastic thyroid cancers being three times more frequent in the ID area when compared to the non-deficient area. Similar findings were also documented in relation to both thyroid cancer incidence rates (IR) observed in periods when iodine prophylaxis was suspended [9] and to an alteration in the frequency rate in the histopathological types of TC in association with ID [9]. A shift from predominance of the more indolent types of papillary TC onto the more aggressive follicular Thyroid carcinoma and anaplastic TC was also observed [9].

Iodine supplementation consistently decreases the incidence of endemic goitre and eradicates IDD [39]. This has been repeatedly demonstrated in many European Countries, such as Finland where a combination of extensive public education was combined with the introduction of a iodised salt program [22] Austria, Denmark, Finland, Iceland, Norway, Sweden, Switzerland and the United Kingdom [22]) as well as in the United States of America (USA) [1] .

None the less, since then, worldwide there have been significant improvements in the correction of iodine deficiency with many countries introducing programs for access to iodized salt as an important measure in the correction of IDD. These measures were coordinated by the establishment in 1985 of the International Council for Control of Iodine Deficiency Disorders (ICCIDD) created with the support of the United Nations Children's Fund (UNICEF), the World Health Organization (WHO) and the Australian Government [40].

Through the work of the ICCIDD, their assistance in the implementation of the recommended programs of correction of IDD and the active role that they had to this effect, a reported 68% of households from areas of the world with previous iodine deficiency had access to iodized salt in 2001. This compared to less than 10% a decade before [40].

The World Health Assembly that was held in 1990, under the auspices of the United Nations, and the subsequently held United Nations World Summit for Children saw the endorsement of resolutions aimed at the elimination of iodine deficiency disorders (IDD) in the world, by all attending countries. The delegations that attended these World Meetings included 71 Heads of State and representatives of 159 Governments [41].This problem was then classified as a major public health problem, that should be tackled with resolve.

Europe has had the highest prevalence of iodine deficiency (59.9%) and has been the area where the proportion of households consuming iodized salt has been shown to be the lowest (27%) [42,1]. This can be attributed to the low priority that many European Countries have given to the salt iodization programs, with most having weak or non-existent National programs for the control of IDD[1].

From Portugal there is no general population data on IN [43,44,45]. The only national study on IN relates to the population group of pregnant women [46] showed iodine deficiency throughout the entire country, with a global nationwide median UIC of 82.5μg/L.

South Africa is documented as having had areas of ID in the years before the introduction in 1995 of legislation requiring all manufactured salt to contain potassium iodate at a higher level than before [47]. This policy has been shown successful in the elimination of ID in South Africa [48].

In population studies, measuring the median urinary iodine concentration (UIC) is the most accurate method of assessing and monitoring the iodine nutrition status done through random spot urine samples measured in μg/L [49]. Iodine deficiency was successfully corrected in S.A. [48] following the introduction of mandatory salt iodization at a higher level than before, in 1995. In the years preceding that, evaluation of iodine nutrition (IN) using UIC was not easily nor routinely available. An alternative method of assessing IN through parameters relating to the thyroid histology pattern has been previously referred to and documented [9].

The aim of the present study is to comparatively evaluate the IN in two different populations with the common characteristic of being iodine deficient at the time of data gathering. Through the awareness of the epidemiological data relating to the problem of iodine deficiency solutions are proposed, that are relatively inexpensive and easy to implement. The ultimate aim is to contribute towards the elimination of ID in Portugal. These solutions could also be useful in the many countries worldwide that are still affected by the problem of ID.

Chapter 2

The environment and iodine deficiency disorders

Iodine the essential component of the hormones produced by the thyroid gland which are essential for mammalian life [1] is only acquired through the diet [49]. Iodine, in the form of iodide is nearly wholly absorbed (>90%) in the stomach and duodenum [50].

Iodine in the form of iodide is widely but unevenly distributed in the earth´ environment, most iodide being found in the oceans [1]. Iodide ions in seawater oxidise forming elemental iodine which is volatile evaporating into the atmosphere to return to the soil with the rain, thus completing the cycle [1].

The iodine cycle in many regions is slow and incomplete with soils becoming deficient in iodine. Iodine deficient soils tend to be more common in inland regions, mountainous areas but can also occur in coastal regions [1,24].

Iodine in soils shows a very broad range of concentrations with the proximity of an area to the sea being likely to exert a strong influence on the iodine content of soils in the area [5]. However, when iodine is strongly sorbed in the soil it is not readily bioavailable. Therefore, the presence of high iodine concentrations in the soil does not necessarily mean that the plants growing in that soil will incorporate large concentrations of iodine. It has in fact been show that there is no correlation between the iodine content of soils and the plants growing on them [51].

Crops grown in iodine deficient soils are low in iodine concentration [1].

The uptake of iodine into plants is accomplished through two mechanisms: through the roots and through leaf stomata, this one being probably the most important [05]. However it has been shown that the bioavailability of iodine in soils is low [51]. For these reasons the iodine content of plants is generally low.

2.1 Water Reservoirs

Surface waters have been regarded as the best index of the local environment´s iodine status, particularly since iodine in water represents the bioavailable form of the element [52]. From a practical point of view waters are much easier to analyse than soils.

Work from the UK and the USA provides a threshold level of 3 µg/L below which an environment could be considered as iodine deficient [52].

A wide range of results is reported for the iodine content of drinking water from <0.1 µg/L to 150 µg/L, the average of the reported results being 4.4 µg/L [52]. As to the level of iodine reported from the so called natural surface waters, rivers and lakes, their ranges are from 1 µg/L to 10 µg/L.

It is considered that, on average, drinking water provides about 10% of the Recommended Dietary Allowance (RDA) [52], which is currently $\geq$ 120 µg/L for schoolchildren (6-12 years), $\geq$ 150 µg/L for adults and $\geq$ 250 µg/L for pregnant or lactating women [53,54]. In regions that are dependent on the local supply for food and without other sources or forms of iodine supplementation, drinking water can provide more than twenty percent of the daily iodine intake [52].

2.2 Sources of iodine intake – Nutrition

The iodine content of most foodstuffs is low the highest being found in sea based nutrition products: sea fish, shellfish of sea origin [55], or other products or supplements originated from the sea.

Fruits and vegetables usually have a very low iodine content, which is also further affected by the method of cooking [55].

In the USA and in some European countries where iodophor disinfectants are used milk may be a significant dietary source of iodine [1,5].

An accurate assessment of food iodine content is extremely difficult for methodological reasons [55]. Therefore most estimates of dietary iodine intake are based on the measurement of the urinary iodine concentration (UIC) [1,55].

2.3 Evaluation of Iodine Nutrition

Iodine nutrition historically has been assessed on clinical criteria by palpation of the thyroid gland and the grading of its size according to an established goitre rate [42]. Although this has been used for many years it is considered a subjective biomarker.

In population studies measuring the median urinary iodine concentration (UIC) is the most accurate method of assessing and monitoring the IN status, through random spot urine samples, in µg/L [49]. The UIC is measured by fast colorimetric method based on the original description method by Sandell and Kolthoff [56] and the median value determined.

In places where the facilities for determining the populations median UIC are not available an alternative method of assessing the population´s iodine nutrition has been through parameters relating to the thyroid histology pattern [9].

2.3.1 Goitre

Thyroid enlargement or goitre is the classic sign of iodine deficiency. Secretion of thyroid stimulating hormone (TSH) tends to increase as a result of insufficient iodine intake [1]. This occurs as part of the process to maximise and optimise the uptake of available iodine. TSH has a thyrotrophic effect stimulating thyroid hypertrophy and hyperplasia.

Initially goitres appear with a diffuse homogeneous enlargement which in time develop into nodular formation [1].

The traditional method for determining thyroid size is the clinical evaluation through inspection and palpation. The size of the thyroid gland tends to change inversely in response to iodine intake with a lag interval that can vary from a few months to years [42].

This is further described in relation to the clinical evaluation of thyroid nodules in section 3.3.

Ultrasonography can also be used as a more precise and objective method. Initially there was disagreement as to criteria and reference values [42], which have now been standardised and are discussed in relation to methods of diagnosis of thyroid nodules in section 3.4.

2.3.2 Urinary Iodine Excretion

The measurement of urine iodine excretion provides the best single objective measurement of the iodine nutritional status of a population [42]. In a review done by Dunn et al [57], the most practical and simple method for determining the UIC was the sensitive colorimetric Sandell-Koltoff reaction [56], in which urine is first acid digested under mild conditions and iodide subsequently determined from its catalytic reduction of cerium ammonium sulphate, in the presence of arsenic acid.

In the current study, on the random spot urine samples obtained, the UIC was measured by the fast colorimetric method based on the original description method of Sandell and Koltchoff [56].

In population studies, measuring the median urinary iodine concentration (UIC) is the most accurate method of assessing and monitoring the iodine nutrition status. This should be done through random spot urine samples measured in µg/L [49].

2.3.3 Histology findings

Thyroid cancer (TC) arises from the epithelial elements of the gland, generally from follicular cells [58]. TC is traditionally divided into two major groups: the differentiated (including the papillary, follicular and medullary tumours) and the undifferentiated (anaplastic) carcinomas. This classification is based on both morphology and clinical features, being strongly supported by advances in molecular studies [58]. The histologic classification of thyroid tumours arrising in follicular cells has evolved considerably in recent decades [59]. The original descriptions were based on the observation that some tumours were predominantly composed of papillae, therefore being called “papillary” carcinomas; others that were prediminantly composed of follicles were labeled “follicular” carcinomas. Tumours that had the two structures were classied as mixed (papillary / follicular) carcinomas.

At the time when “papillary” and “follicular” carcinomas were coined goitre was endemic in many parts of the world and the pathologists (mostly German and Swiss) [59], noticed that the follicular carcinomas were morphologically very similar to the nodules or adenomas od the nodular adenomatous endemic goitre (EG). With the measures taken for the elimination of ID in many parts of the world EG has slowly been eliminated. Its disappearance has also been accompanied by changes in the relative frequencies of the thyroid carcinomas with the frequency of the more aggressive anaplastic and follicular carcinomas decreasing and papillary carcinoma becoming predominant, therefore with documented alterations in the frequency rates being observed in relation to variations in the iodine nutrition of the population [9].

Another important change in the original concept of the classification of thyroid cancer has been the realization of the mode of spread of these tumours and their nuclear morphology should also be noted [60].

Papillary carcinoma cells typically penetrate the stroma in multiple small clusters that create a marked fibroplastic reaction, that never the less does not stop them. Papillary carcinoma cells cells usually form papillary structures. The nuclei of these cells are of the typical ground-glass appearance (“Orphan Annie”). Ultrastructural studies revealed that the typical ground glass appearance is due to multiple invaginations of the cytoplasm into the space that on light microscopy corresponds to the nucleus [59].

By contrast , follicular carcinomas do not produce marked fibroplasia, but rather compress the neighbouring parenchyma causing pseudocapsules, which generally contain telangiectatic vessels that are frequently invaded by tumour cells [59]. This is one of the basis for the tumour behaviour of follicular carcinomas that can spread by blood born distant metastasis.

The above morphologic criteria have been integrated and replaced the old ones as part of the distinction between papillary and follicular carcinomas. The change from one set of criteria to the other has however not been uniform, with some pathologists still using the old criteria [59].

Papillary thyroid cancer (PTC) is the predominant form of thyroid cancer (TC) where iodine sufficiency exists [11,12] with papillary to follicular (P:F) ratios of 3.4-6.0:1 reported from the USA [12]. By contrast, in iodine deficient areas follicular thyroid cancer (FTC) tend to predominate [11,12,13]. Therefore, geographical areas where ID prevails display an inversion in the usual frequency ratio of P:F thyroid cancer. They also have a relative increase in the incidence of anaplastic carcinomas [9,13].

2.3.4 Thyroglobulin

Thyroglobulin (Tg) is synthesized only in the thyroid and is the most abundant intrathyroidal protein. In iodine sufficiency small amounts of Tg are secreted into the circulation. In areas of endemic goitre serum Tg increases due to thyroid cell hyperplasia and TSH stimulation. Intervention studies evaluating the potential of Tg as an indicator of response to iodine supplementation have shown that Tg concentrations fall rapidly with iodine supplementation. Tg is a more sensitive indicator of iodine repletion than TSH or T4.

Limitations in the use of Tg as an indicator of iodine nutrition are the need for concurrent measurement of anti-thyroglobulin antibodies to avoid potential underestimation of TG. Other problems are large inter-assay variability and a poor reproducibility [61]

Chapter 3

Thyroid Nodules – Methods of Diagnosis: Benign and Malignant Characteristics. Iodine Deficiency and Thyroid Cancer.

Thyroid nodules are relatively common in adults. They are discovered in clinical practice either because of patient concerns or during a clinical examination of the neck. They are sometimes discovered incidentally in the course of various imaging procedures [62].

Their estimated prevalence depends on the identification method ranging from 4% to 7% of the adult population on palpation alone [62], whereas on ultrasound (US) it ranges from 20% to 76% of the adult population [62]. The reported frequencies on US correlate with the prevalence reported at autopsy that range between 8.2% and 65% [29,36].

3.1 Thyroid Anatomy

The thyroid gland consists of two lobes (right and left) and a midline isthmus joining them. The small pyramidal lobe, of variable size is present in between 30% and 60% of people. It results from the persistence of the embryologic thyroglossal duct and is commonly located at the junction of the isthmus with the left lateral lobe, as displayed in Figure 1.

The principal blood supply of the thyroid gland derives from the inferior thyroid artery, a branch of the thyrocervical trunk deriving from the subclavian artery and from the superior thyroid artery which is the first branch of the external carotid artery. The venous drainage of the thyroid gland is done through the superior and middle thyroid veins which empty into the internal jugular vein and the inferior thyroid veins emptying into the brachiocephalic veins.

Each lobe is located in the space between the trachea and the oesophagus medially and the carotid sheath laterally. The connections of the thyroid gland to the pretracheal fascia, which splits to invest the gland, makes the thyroid move up and down on swallowing. The fascia which forms a surgical capsule sends fibrous septa into the gland substance, dividing it into numerous lobules. Each lobule has 30 to 40 follicles. These are secretory and storage units formed by a circle of cuboidal epithelial cells that secrete colloid and the thyroid hormones T3 and T4 into the centre of the follicle, Figure 2.

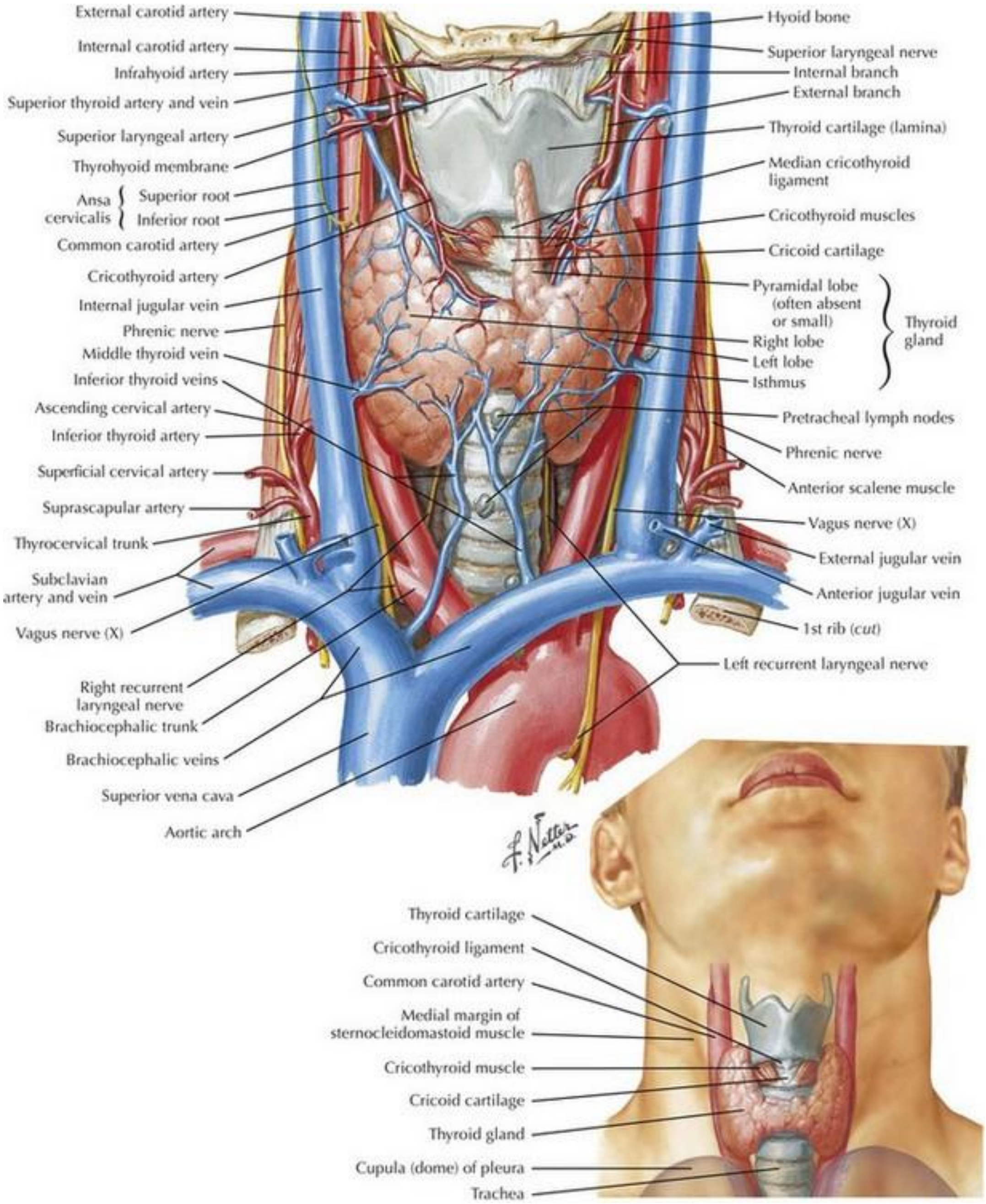


Figure 1. Anterior view of the thyroid gland displaying its relations to the adjacent structures. Adapted with permission from McHenry CR Thyroidectomy and Parathyroidectomy; Chapter 3; 21-36 https://clinicalgate.com/thyroidectomy-and-parathyroidectomy-2/

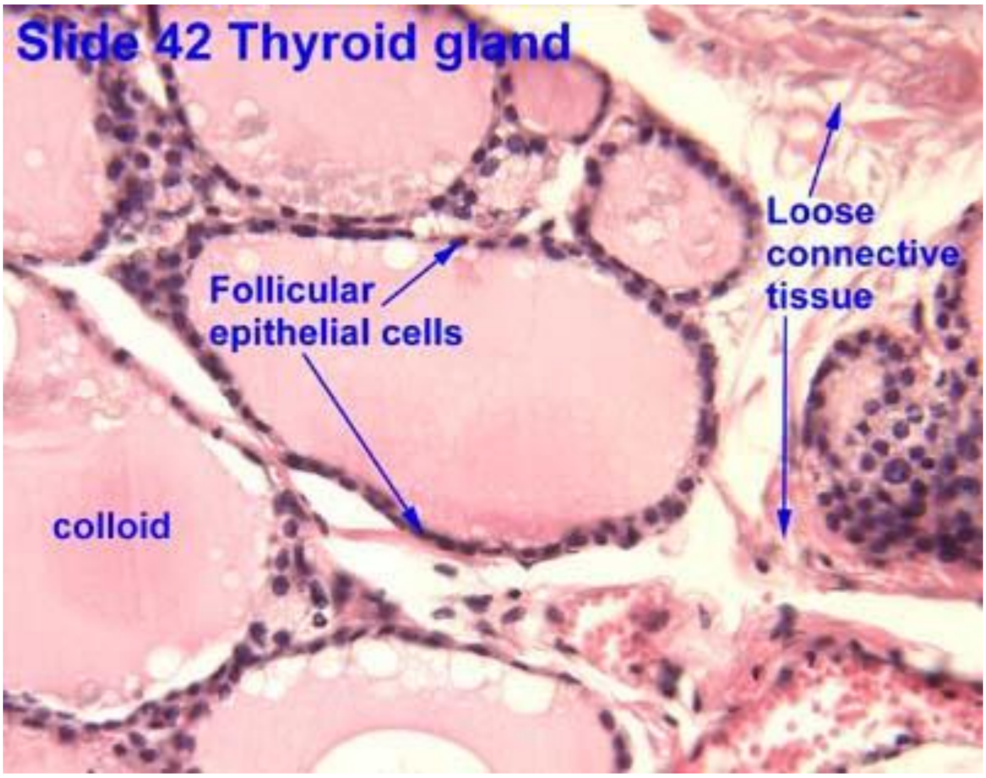


Figure 2. Inset of thyroid gland histology displaying colloid filled thyroid follicles. Each follicle surrounded by a delicate layer of loose connective tissue. A capillary is also displayed. Adapted with permission from Histology-Endocrine http://www.ouhsc.edu/histology/Glass%20slides/42_03.jpg Copyright © 2016 The Board of Regents of the University of Oklahoma.

3.2 Physiology of the Thyroid Gland

The thyroid hormones T3 and T4 are produced by the thyroid follicular cell (thyrocyte) and stored in the colloid, in thyroglobulin, an iodinated glycoprotein. Thyroglobulin stores are dependent on adequate dietary iodine intake since the synthesis of T3 and T4 requires iodine.

Synthesis within the thyroglobulin complex is dependant on a series of enzymes, in distinct steps namely: 1) the trapping of inorganic iodide from the blood; 2) the oxidation of iodide to iodine; 3) the binding of iodine to tyrosine to form iodotyrosines; 4) the coupling of monoiodotyrosines and di-iodotyrosines to form T3 and T4 [63].

Under TSH influence, when thyroid hormones are required thyroglobulin is resorbed into the thyrocyte where thyroglobulin is broken down. The hormones T3 and T4 result and enter the blood, where they become bound to serum proteins: albumin, thyroxine binding globulin (TBG) and thyroxine binding prealbumin (TBPA). The small amount of hormone that remains free in the serum is biologically active [63].

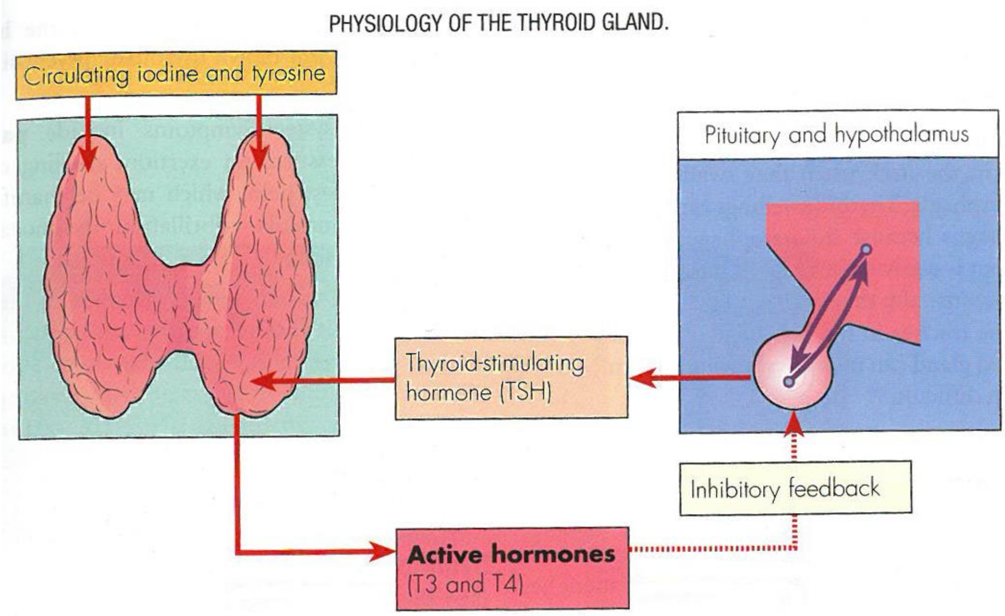


Figure 3. Physiological principles of thyroid and the pituitary – hypothalamic axis.
Adapted with permission from Browse´s Introduction to the Symptoms and Signs of Surgical Disease, Fourth Edition 2005.

3.3 Clinical Evaluation

There are two main types of symptoms and signs connected with the thyroid pathology: those related to the swelling of the neck and those related to the endocrine activity of the gland. Understanding the anatomy and physiology related to the thyroid gland is essential to enable an accurate assessment.

The clinical evaluation includes a good medical history that has to be thorough and oriented to the problems related to thyroid. This is complemented by an examination aiming at eliciting the relevant physical signs. This should include attention to the development of the neck lump; the presence of dyspnoea and its characteristics; discomfort or difficulty swallowing; hoarseness; symptoms or signs of either hyperthyroidism or hypothyroidism.

A careful examination of the neck following the routine of inspection, observance of swallowing, examining the thyroid gland with the characteristics of any enlargement or nodule as well as to its characteristics. Examination of the neck for possible lymph nodes is important.

As part of the clinical examination directed at thyroid pathology feeling the pulse and its characteristics including tachycardia or bradycardia will direct the clinician towards a functional abnormality of hyper or hypothyroidism. Examining the as to whether they are moist, warm or sweaty is important in the diagnosis of hyperthyroidism. Equally important is the examination of the eyes and ocular movement.

In thyroid nodular pathology the history and examination should focus on detecting features that are suggestive of possible malignancy.

There are certain characteristics that increase the likelihood of a thyroid nodule being a thyroid carcinoma. Some of these are listed in Figure 4.

CLINICAL FINDINGS RELATED TO RISK OF CARCINOMA IN A THYROID NODULE

History of childhood head or neck therapeutic irradiation, total body irradiation or exposure to ionizing radiation from fallout (e.g. Chernobyl).

Family history of thyroid cancer or of a syndrome associated with thyroid cancer (e.g. familial adenomatous polyposis, Cowden´s syndrome, multiple endocrine neoplasia 2)

Age <20 or >65

Men > women (in terms of proportion of nodules that are carcinoma)

History of other cancer , especially kidney, breast, lung or melanoma

Vocal cord paralysis

Abnormal lateral cervical adenopathy

Firm nodule fixed to surrounding tissue

Figure 4. Clinical findings related to risk of carcinoma in a thyroid nodule.**Adapted with permission from: "Werner & Ingbar´s The Thyroid A Fundamental and Clinical Text, Tenth Edition, 2013; Chapter 49, Clinical evaluation and management of thyroid nodules.**

A spectrum of diagnostic studies are available to assist in the evaluation of a thyroid nodule. These include:

3.4 Ultrasonography

Sonographic criteria have been established that aid in determining which nodules should be considered for further cytological investigation. These are: the finding of microcalcifications suggestive of malignancy (tiny,punctate hyperechoic foci), irregular or microlobulated margins, marked hypogenicity, as well as the nodule shape/dimension ratio: in that the nodule being taller than wider indicates a higher likelihood of it being malignant [32]. These criteria have been shown, in a study of 155 nonpalpable thyroid nodules that were subjected to Fine Needle Aspiration Biopsy (FNAB), to have a sensitivity of 93.8%, a specificity of 66% and an accuracy of 74.8% [32]. Figure 5. summarizes the diagnostic relevance / index for each individual sonographic criteria of malignant nodules.

TABLE 1 Diagnostic Index for Individual Sonographic Criteria of Malignant Thyroid Nodules

Sonographic Characteristics	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Accuracy (%)
Microcalcification	29/49 (59.2)	91/106 (85.8)	29/41 (70.7)	91/114 (79.8)	120/155 (77.4)
Irregular or microlobulated margin	27/49 (55.1)	88/106 (83)	27/45 (60)	88/110 (80)	115/155 (74.2)
Marked hypoechogenicity	13/49 (26.5)	100/106 (94.3)	13/19 (68.4)	100/136 (73.5)	113/155 (72.9)
More tall than wide	16/49 (32.7)	98/106 (92.5)	16/24 (66.7)	98/131 (74.8)	114/155 (73.5)

Note—Most malignant nodules had multiple sonographic findings suggestive of malignancy (mean number of suspicious findings per malignant nodule, 2.6). The accuracy of each sign was 72.9–77.4%, but the sensitivity was low, ranging from 26.5% to 59.1%.

Figure 5. Diagnostic Index for Individual Sonographic Criteria of Malignant Thyroid Nodules Adapted with permission from Kim E-K, et al. Am J Roentgenol 2002; 178:687-691.

The absolute and percentage figures as to the individual sonographic criteria having been present in benign and malignant nodules in the 155 prospectively evaluated and classified were also provided (Figure 6.)

TABLE 2 Sonographic Findings in 155 Malignant or Benign Thyroid Nodules

Sonographic Characteristics	Malignant Nodules (%) (*n* = 49)	Benign Nodules (%) (*n* = 106)
Microcalcification	29 (59.1)	15 (14.2)
Irregular or microlobulated margin	27 (55.1)	28 (26.4)
Marked hypoechogenicity	13 (26.5)	6 (5.6)
More tall than wide	16 (32.7)	8 (7.5)

Note—All findings appear to be statistically significant (p <0.5, using the chi-square test).

Figure 6. Sonographic Findings in 155 Malignant or Benign Thyroid Nodules. Adapted with permission from Kim E-K, et al. Am J Roentgenol 2002; 178:687-691.

The Society of Radiologists in Ultrasound (USA) issued their Guidelines [33], recommendations for FNAB. These criteria state that ultrasound (US) guided FNAB on any nodule 1cm in diameter or larger with microcalcifications, any nodule 1.5cm or larger that is solid or has coarse calcifications within the nodule.

Subsequently the Society of Radiologists in Ultrasound (USA) issued their consensus statement Guidelines [33] on which nodules should be subjected to ultrasound (US) guided FNAB and which should not.

US Features Associated with Thyroid Cancer

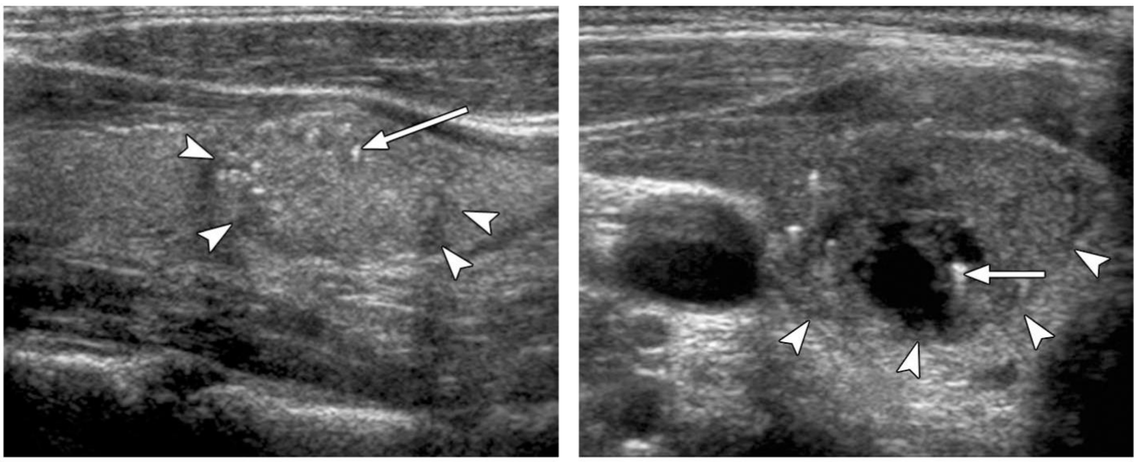
US Feature*	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Microcalcifications (1–5)	26.1–59.1	85.8–95.0	24.3–70.7	41.8–94.2
Hypoechogenicity (2–5)	26.5–87.1	43.4–94.3	11.4–68.4	73.5–93.8
Irregular margins or no halo (2–5)	17.4–77.5	38.9–85.0	9.3–60.0	38.9–97.8
Solid (4–6)	69.0–75.0	52.5–55.9	15.6–27.0	88.0–92.1
Intranodule vascularity (3, 6)	54.3–74.2	78.6–80.8	24.0–41.9	85.7–97.4
More tall than wide (2)	32.7	92.5	66.7	74.8

* Numbers in parentheses are reference numbers.

Figure 7. Ultrasound features associated with thyroid cancer. Adapted with permission from Frates MC, et al. Radiology 2005;237:794-800.

The American Association of Clinical Endocrinologists has also produced their Guidelines jointly with the Italian Association of Endocrinologists and the European Thyroid Association [34].

Displayed below, in figure 8, are examples of ultrasound (US) calcifications with a.- features of malignancy and of b.- colloid crystals in a benign nodule.



a. b.

Figure 8. (**a.**) Sagittal US image of a nodule (arrow-heads) containing multiple fine echogenicities (arrow) with no comet-tail artefact. These are highly suggestive of malignancy. FNA and surgery confirmed a papillary carcinoma. **(b.)** Transverse US image of nodule (arrowheads) containing cystic areas with punctate echogenicities and comet-tail artefact (arrow) consistent with colloid crystals in a benign nodule. Adapted with permission from:

Frates MC, et al. Management of Thyroid Nodules at US: Society of Radiologists in Ultrasound Consensus Conference Statement. Radiology 2005; 237:794-800.

3.4.1 Thyroid ultrasound elastography (USE)

Ultrasound elastography is a new emerging technique that is non-invasive and measures tissue elasticity using an ultrasound probe. The procedure is based in the fact that soft tissue structures can be compressed more than the hard ones and that malignant tumours generally tend to consist of harder tissue when compared to benign lesions [64].
With USE elasticity is assessed using a colour scale that goes from red (soft areas), to yellow and green (medium elasticity), to blue (hard lesion). It is hoped that with the future development of this technique with newer more sensitive colour display devises this technique might provide good data regarding the sensitivity and specificity of this technique.

3.5 Radionuclide Imaging

Radionuclide imaging is done for diagnostic purposes using mainly technetium 99. Iodine (either I125 or I131) can also be used mainly when ablation therapy is contemplated.

The main limitation of this technique lies in it not being able to distinguish benign from malignant nodules and can only estimate a probability of malignant disease on the basis of the functional status of a nodule.

From reviews that were done malignant disease was found on subsequent histology in 16% of the cold nodules, 9% of the warm nodules and 4% of the hot nodules [64].

3.6 Cytology

The diagnostic investigation of choice for a worrying thyroid nodule or for an unexplained thyroid nodular pathology is Fine Needle Cytology Biopsy (FNB), which enables cytological evaluation and frequently provides a cytological diagnosis [11]. The previously commonly used term of fine needle aspiration biopsy (FNAB), should be superseded by the more general and therefore perhaps more appropriate term of FNB [11]. This particularly since it has been demonstrated in several publications that aspiration by negative pressure is not only, not necessary [11]. but also that the non-aspiration fine needle cytology (NAFNC) technique can produce statistically significantly superior, more diagnostic, results when compared to the FNAB technique, the specimens being less haemorrhagic and therefore having more diagnostic cellular material [65].

In the non-aspiration ("nonsuction") technique, described originally by the author of the current study (the first publication on this new technique) [65], "the needle is gripped like a pencil, which facilitates precise needle placement for small nodules", and allows the needle to be moved in and out over a few millimetres and rotated. "This combined motion uses the bevel of the needle for cutting, which frees cells that flow into the needle by capillary action" [11]. With this technique fluid material can usually be seen entering the hub of the needle, which is subsequently expelled onto a glass slide and prepared in the usual manner for cytological examination.

This technique is also referred to, in some publications, as fine needle capillary cytology, the term that was used by the original author of this new technique, in the initial presentations made at academic meetings in South Africa. Subsequently this term of "capillary cytology" was abandoned by this author for fear of giving rise to confusion as to what was being sampled and the original publication on this new technique used the designation "nonaspiration fine needle cytology" [65].

In the cases where the cytology result is equivocal, where it raises the possibility of a neoplasm or in patients where the clinical picture would indicate that a surgical procedure would be advisable, histopathological examination is usually obtained.

It is important to realize that the majority of patients that present with a thyroid nodule end up being proven to have a benign nodule and are not routinely recommended to have surgery [11]. For these patients the recommended management protocol should be observation and reassessment. If clinically or on ultrasound criteria indicated repeat FNB should be performed.

3.7 Histology

Thyroid cancer (TC) arises from the epithelial elements of the gland, mostly from the thyroid follicular cell [58]. TC is traditionally divided into two major groups: the differentiated (including the papillary, follicular and medullary tumours) and the undifferentiated (anaplastic) carcinomas. This classification is based on both morphology and clinical features, being strongly supported by advances in molecular studies [58]. The histologic classification of thyroid tumours arising in follicular cells has evolved considerably in recent decades [59]. The original descriptions were based on the observation that some tumours were predominantly composed of papillae, therefore being called "papillary" carcinomas; others that were predominantly composed of follicles were labelled "follicular" carcinomas. Tumours that had the two structures were classified as mixed (papillary / follicular) carcinomas.

At the time when "papillary" and "follicular" carcinomas were coined goitre was endemic in many parts of the world and the pathologists (mostly German and Swiss) [59], noticed that the follicular carcinomas were morphologically very similar to the nodules or adenomas of the nodular adenomatous endemic goitre (EG). With the measures taken for the elimination of ID in many parts of the world EG has slowly been eliminated. Its disappearance has also been accompanied by changes in the relative frequencies of the thyroid carcinomas with the frequency of the more aggressive anaplastic and follicular carcinomas decreasing and papillary carcinoma becoming predominant, therefore with documented alterations in the frequency rates being observed in relation to variations in the iodine nutrition of the population [9].

Another important change in the original concept of the classification of thyroid cancer has been the realization of the mode of spread of these tumours and their nuclear morphology should also be noted [60].

Papillary carcinoma cells typically penetrate the stroma in multiple small clusters that create a marked fibroblastic reaction. The cells usually form papillary structures. The nuclei of the papillary carcinoma cells are of the typical ground-glass appearance ("Orphan Annie"). Ultrastructural studies revealed that the typical ground glass appearance is due to multiple invaginations of the cytoplasm into the space that on light microscopy corresponds to the nucleus [59].

By contrast, follicular carcinomas do not produce marked fibroplasia, but rather compress the neighbouring parenchyma causing pseudocapsules which generally contain telangiectatic vessels that are frequently invaded by tumour cells [59]. This is one of the basis for the tumour behaviour of follicular carcinomas that can spread by blood born distant metastasis.

The above morphologic criteria have been integrated and replaced the old ones as part of the distinction between papillary and follicular carcinomas. The change from one set of criteria to the other has however not been uniform, with some pathologists still using the old criteria [59].

Papillary thyroid cancer (PTC) is the predominant form of thyroid cancer (TC) where iodine sufficiency exists [11,12] with papillary to follicular (P:F) ratios of 3.4-6.0:1 reported from the USA [12]. By contrast, in iodine deficient areas follicular thyroid cancer (FTC) tend to predominate [11,12,13]. Therefore, geographical areas where ID prevails display an inversion in the usual frequency ratio of P:F thyroid cancer. They also have a relative increase in the incidence of anaplastic carcinomas [9,13].

Chapter 4

Material and Methods

4.1- Material

4.1.1 Study design

This study was approved by the ethics committees of the Faculty of Health Sciences of the University of Beira Interior and of Centro Hospitalar Cova da Beira (Hospitals of Covilhã and Fundão), as well as by the Committee for Research on human subjects of the University of the Witwatersrand/ Baragwanath Academic Hospital.

The ultimate aim of the current study is to contribute towards the elimination of ID in Portugal and in countries and regions in which this problem is still prevalent, highlighting it´s serious negative consequences and proposing possible solutions that are easily applicable and affordable.

Towards this aim, a comparative evaluation of the iodine nutrition of the population from the region of Beira Interior (BI) in Portugal, a country from which there is no general population data available [4,43,66], and of the available parameter of assessment of iodine nutrition from the population from the Johannesburg (JHB) region, reflected by the predominantly black African population seen at the largest referral Hospital in South Africa (SA), Baragwanath Hospital (BH) was sought. The available thyroid histopathology pattern from the JHB, S.A. area, relating to the period between January 1984 and December 1988, prior to the introduction of mandatory salt iodization in 1995 , was used [67].

In places where the facilities for determining the populations median UIC are not available an alternative described method of assessing the population´s iodine nutrition has been through parameters relating to the thyroid histology pattern [9].

The introduction of mandatory salt iodization in South Africa in 1995 [47] has been proven successful in eliminating ID [48]. Whilst this noteworthy success demonstrates an easy to follow example for regions that still experience the problem of ID, it also means that UIC data demonstrating the previous ID status in South Africa would have had to be obtained from publications that preceded this measure.

The evaluation of UIC was at that time not generally performed. The evaluation of the histopathology pattern and it´s characteristics is an alternative and complementary way to measurements of UIC in demonstrating ID, as has been highlighted in previous studies [9].

The study region for BI to be evaluated was defined as that situated beyond the mountain range of Serra da Estrela, a mountainous range stretching across 115 km, with the highest peak at 1993 metres above sea level [68]. The area of JHB is situated in a plateau at approximately 1,750 meters of altitude [69].

4.1.2 Cytology

Cytological evaluations are reported as being relatively inaccurate when compared with the subsequent histological diagnoses [70] making them unsuitable as a tool for epidemiological purposes. As such they tend to be used as a screening tool in patient management, as in the present study, guiding as to which patients should be submitted to surgery and influencing its extent.

4.1.3 Histology

Diagnosis evaluation by Histology

Patients that had histology available had been selected for thyroid surgery, in both areas, based on cytology criteria, ultrasound findings or clinical indications. These included family history of thyroid malignancy, an enlarging nodule or the presence of neck lymphadenopathy. A representative and illustrative sample of the thyroid nodular pathology in each of these areas was sought so as to enable a comparative evaluation of the patterns of pathology in these two areas.

Histology reports relating to patients residing in the inland region of BI, performed during the period between January 2002 and December 2008 were evaluated. The 6 year time period was arbitrarily chosen, aiming at obtaining a representative sample of the thyroid nodular pathology in the area. All reports from the local major referral Hospitals linked to the University of Beira Interior, (the only State University in this region), namely the Hospitals of Covilhã and Fundão, were obtained. This data was complemented with histology reports from the Portuguese Oncology Institutes of the three main nationwide centres of Lisbon, Oporto and Coimbra (the latter being the main Oncology referral centre for the region of BI), from patients that provided a residential address from the study area of BI.

Thyroid histology reports evaluated from the JHB area related to patients that had presented to the BH thyroid clinic in JHB during the 5 year period between January 1984 and December 1988 [67], this corresponding to a time preceding the introduction of mandatory salt iodation in South Africa [47].

The standard method for general histology with haematoxylin and eosin staining (H&E) and criteria according to the WHO revised classification of thyroid tumours was employed in both study areas, histology examinations having been performed by specialist pathologists with an interest in thyroid pathology at Academic Pathology Centres. The diagnostic criteria followed at both centres: CEDAP, linked to the University Hospital of Coimbra (Centro Hospitalar e Universitário de Coimbra) and at the South African Institute for Medical Research (S.A.I.M.R.), in Johannesburg, connected to the University of the Witwatersrand, followed the same diagnostic criteria, as defined by the W.H.O.

Although the time periods for data collection were different, they both correspond to periods when ID prevailed in each study area [46,47].

4.1.4 Urinary Iodine Excretion - Population sample and measurements

In population studies measuring the median urinary iodine concentration (UIC) is the most accurate method of assessing and monitoring the IN status, through random spot urine samples, in µg/L [49].

Urinary Iodine Concentration measurements were not easily available nor routinely done in Johannesburg, South Africa, during the 5 year study period between January 1984 and December 1988.

For that reason the alternative, previously described method of assessing the population´s iodine nutrition through parameters relating to the thyroid histology pattern [9] was used.

From the study region of BI Urinary iodine excretion was measured from a general population sample of 214 volunteers (131 females and 83 males), with ages ranging from 8 to 97 years (mean 51.5 years ± SD 20.74 years), residing in the study area of BI, that had not had thyroid surgery and were not taking thyroid medication or supplements. These samples were collected over a 2 year period, in late 2012, 2013 and early 2014. Samples were obtained from people residing in the region of Beira Interior on a random unselected sampling basis, from people from different walks of life, following informed consent principles. Exclusion criteria were: known thyroid pathology or thyroid medication; medication that might interfere with iodine absorption or metabolism; vitamin supplements that might contain iodine or following atypical diets for the Portuguese population (i.e. strict vegetarians or vegans).

Random spot urine samples were obtained, UIC measured by fast colorimetric method based on the original description method by Sandell and Kolthoff [56] and the median value was determined.

4.1.5 Study Population

According to the currently available population census of 2011 [71], this selected sample area of Beira Interior has a population of approximately 232,000 inhabitants. The male to female ratio in this region is approximately equal until to the age of 65. After the age of 65, reflecting the fact that males have a shorter life expectancy there is a slight female preponderance. To the totals referred to in this publication of the Bulletin of Geography, which represent the summing up of the population from the three regions that are to the east of the mountain range of Serra da Estrela: Cova da Beira (the areas of Covilhã, Fundão and Belmonte), Beira Interior North and Beira Interior South, the population of Castelo Branco totalling 55,708, which is in the southernmost area of Beira Interior South was deducted from this total, as this specific area lies further South of the mountain range of Serra da Estrela and the plain area where it lies is no longer an area that is “shielded by” this mountain range. Patients originating from this area were similarly not entered into the database for statistical analysis.

4.2 – Methods

4.2.1 Cytology

Fine needle cytology biopsy (FNB), the investigation of choice for a thyroid nodule, can be done using the aspiration technique (FNAB) or using the newer technique, without the use of aspiration, Fine needle nonaspiration cytology, applying the principle of the capillary of physics [65]. For this reason this technique is often referred to, in the literature, as the method of capillary cytology.

4.2.1.1 Fine Needle Aspiration Biopsy

The technique used for fine needle aspiration biopsy (FNAB) was the one that is generally performed at most centres worldwide, based on the original description by Lowhagen et al. from the Karolinska Institute in Stockholm [72]. This procedure should be performed by an experienced clinician, who has been trained in examining the thyroid gland as well as in the technique of performing FNAB. Details on the way the procedure is done should be described to the patient and the patient’s questions relating to it are answered in a way as to minimize anxiety and concern. The patient should be informed that usually more than one pass of the needle is done in order to maximize the chances of obtaining a diagnostic sample. Possible complications, even though rare and minor in experienced hands, should also be mentioned. All these details are important, not only to be able to obtain the cooperation of the patient but also to obtain an informed consent.

The patient is placed comfortably in the supine position on a flat examination couch, with the neck fully extended and stabilized with a pillow placed under the shoulders. This, by now conventional FNAB technique for the collection of cytological samples in the evaluation of thyroid nodules, consists in the introduction of a fine needle, usually a 22-25 gauge needle, attached to a 10 c.c. syringe, into the nodule that needs to be evaluated, which is fixed by the operator's non dominant hand, (Figure 9) . The use of a mechanical syringe holder, such as the Cameco Syringe pistol (Precision Dynamics Corporation, Burbank, CA), allows one hand to be free to stabilize the nodule that is to be aspirated.

Figure 9. FNAB performed with a mechanical syringe holder with the nodule isolated and stabilized. From: Santos JEC 1995. The role of Fine Needle Cytology in Thyroid Disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

The needle is moved in different directions and at different angles within the nodule whilst suction is applied onto the syringe by pulling the handle. This manoeuvre is used in an attempt to increase the representativeness of the cells to be sampled from within the nodule and therefore to increase the diagnostic yield of this cytological investigation (Figure 12). In this way an attempt is made to have as representative a sample as possible of the cellular material within the nodule and therefore to maximize the potential of the diagnostic accuracy and to eliminate false negatives. The aspiration / suction, is stopped by releasing the plunger of the syringe before the needle is withdrawn from the nodule. In doing so the vacuum and therefore the suction from the syringe is eliminated before the needle is withdrawn from the nodule, thus allowing for a pressure equilibrium to be reached and the vacuum / suction pressure to be eliminated.

4.2.1.2 Fine Needle Nonaspiration (Capillary) Cytology

The technique of fine needle nonaspiration cytology [65], which is reported as providing specimens that are less haemorrhagic and potentially with a diagnostic superior quality, is performed in a similar manner to the technique of FNAB except for not using a syringe nor suction. This technique, displayed in Figure 10, is based in the principle of physics of capillary tension (Figure 11). The needle is gripped like a pencil allowing it to be moved both in and out a few millimetres, as well as rotating it, similar to the technique used in FNAB (Figure 12). This combined motion uses the bevel of the needle cutting and dislodging cells that flow into the needle hub by capillary action. Colloid and cellular material can usually be seen entering the hub of the needle. Samples obtained using this technique are less likely to be diluted with blood compared to samples obtained using suction. If the nonaspiration method does not yield a satisfactory sample or if the nodule is cystic, a 10 c.c. syringe is then used.

After needle withdrawal a 10 c. c. syringe filled with 3 to 4 mL is used to force the cellular material onto a glass slide. Preparation of the slide is exemplified below in Figures 13 and 14.

Figure 10. Demonstration of Fine Needle Nonaspiration Cytology. The needle is gripped like a pencil, moving it in and out and in different directions within the nodule being sampled. From: Santos JEC, Leiman G. Nonaspiration Fine Needle Cytology- Application of a New Technique to Nodular Thyroid Disease. Acta Cytol 1988; 32:353-356, S Karger AG.

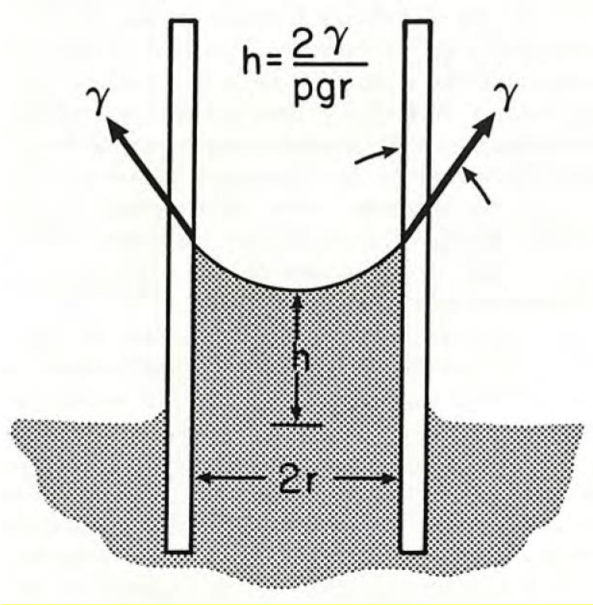


Figure 11. The principle of capillary tension. The ascent of fluid into a narrow channel is governed by the formula h = 2γ/pgr, where h is the height attained, γ is the fluid surface tension, p is the density of that fluid, g is the gravity and r is the radius of the tube.

From: Santos JEC, Leiman G. Nonaspiration Fine Needle Cytology- Application of a New Technique to Nodular Thyroid Disease. Acta Cytol 1988; 32:353-356, S Karger AG.

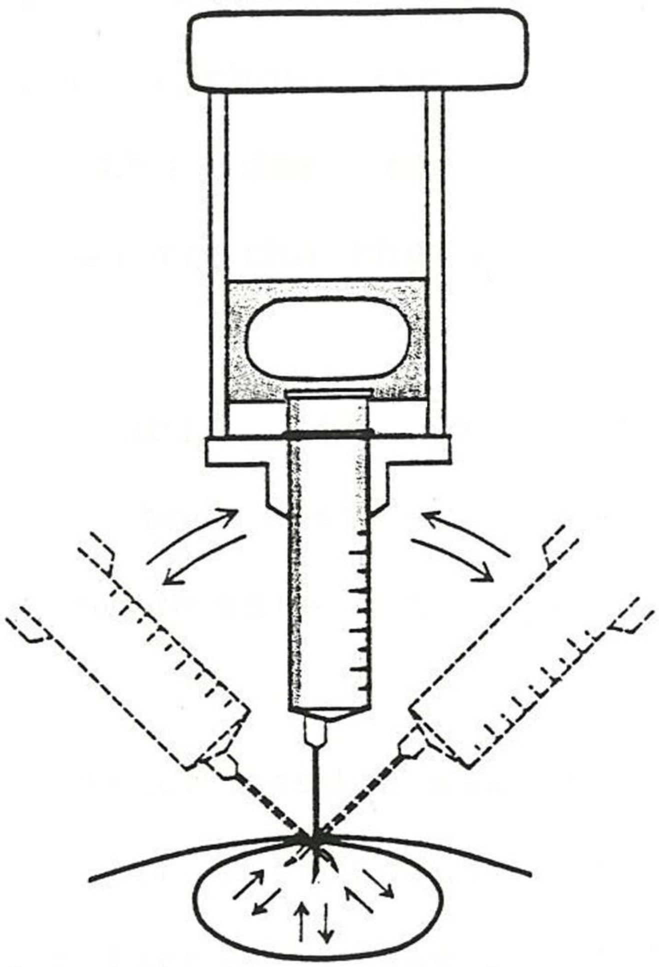


Figure 12. FNAB performed with the needle being moved in different directions within the nodule to be sampled whilst aspiration is applied. From: Santos JEC 1995 The role of fine needle cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

This method is based on the original description by Lowhagen et al. [72] as well as in the modified technique that was described by the main author of this study in his Thesis: The role of Fine Needle Cytology in thyroid disease [67].

The use of a syringe holder, such as the one demonstrated in the drawings of Figure 9 and Figure 12, provides for a more controlled and steadier grip and therefore provides an advantage for the goal of achieving a cytological sample that is more likely to be more representative and more likely to reflect the correct diagnosis of the thyroid nodule being investigated.

Predominantly liquid material arising from the aspiration of a thyroid cyst should be collected into a tube that is subsequently centrifuged. The sediment obtained in this way is then placed onto a glass slide and prepared in the same way as the droplet obtained by FNAB.

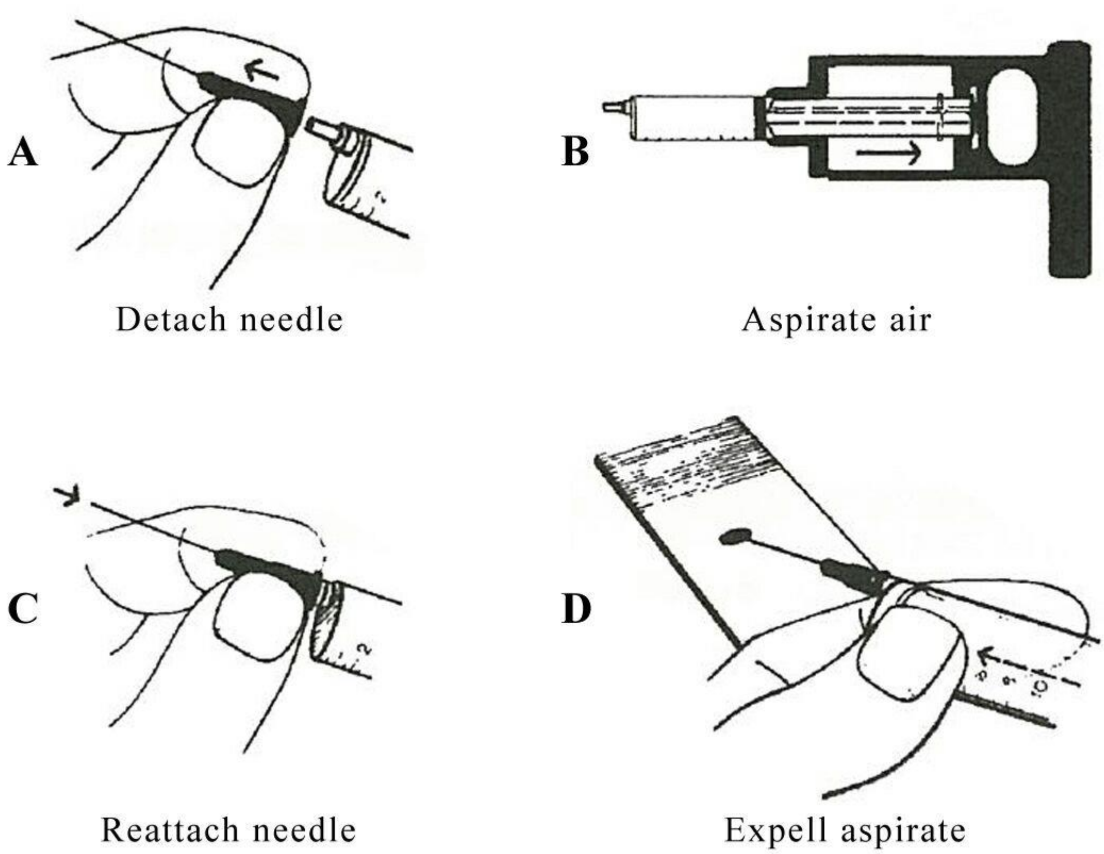


Figure 13. The aspirated material is transferred onto the glass slide. From: Santos JEC 1995. The role of fine needle cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

The droplet is then gently squeezed with another glass slide and by using a drawing movement towards the operator a thin smear of usually colloid and cellular material is obtained (Figure 14). The author of this Thesis finds it preferable that the slides are positioned parallel to each other, as is shown in Figure 14. In this way two equivalent good quality cytology slides are obtained for cytological evaluation.

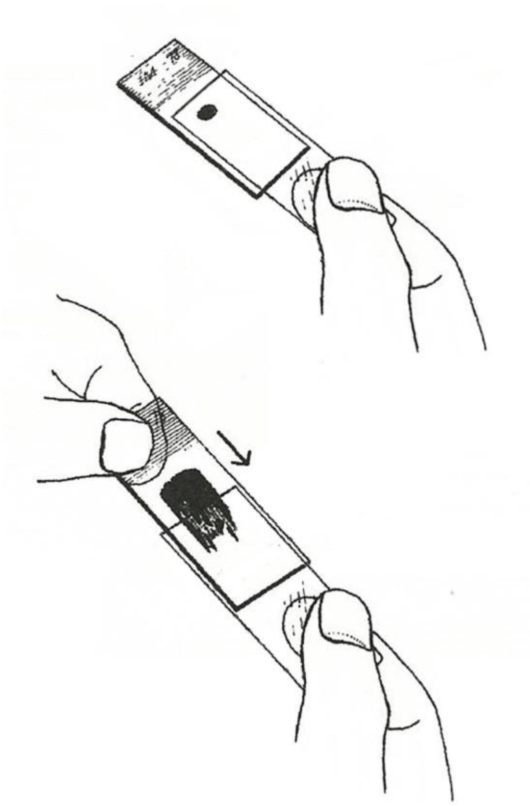


Figure 14. Preparation of the smear which is then either immediately fixed with an alcohol based spray, for subsequent staining according to the method of Papanicolau or air dried, the slide being labelled as such, and subsequently stained by the May-Grunwald-Giemsa method. From: Santos JEC 1995 The role of fine needle cytology in thyroid disease. Thesis (Master of Medicine) University of the Witwatersrand, Johannesburg, South Africa.

The parallel positioning of the glass slides has been found to contribute towards achieving better quality slides and therefore, more diagnostic smears. It is important that the droplet of aspirated material that is deposited onto the glass slide is smeared immediately after being expelled, since air-drying of the droplet will result in crushing and consequent distortion of the cells when they are spread onto the slide, therefore contributing to a non-diagnostic cytological report.

As usually only a small droplet is deposited onto each glass slide more than one slide is produced for cytological screening by the cytology technician and subsequent examination and reporting by the Specialist cytopathologist. Whilst the slides are usually mostly fixed with an alcohol based fixative at least one slide should, ideally, be air-dried and labelled as such, for subsequent staining with the May-Grunwald-Giemsa (MGG) stain. In this way it is felt that the diagnostic yield is maximized since alcohol fixation and Papanicolau staining provides better preservation of the cellular membrane and nuclear structure whereas air-drying and MGG staining provides for better staining of cytoplasmic details and of the extracellular substances such as colloid and precipitated material [67].

Being able to obtain good quality diagnostic slides in cytopathology is related to the experience of the clinician that is performing the F.N.C. sampling procedure. It also requires the use of the correct slide smearing and fixing techniques. There is a direct correlation between the level of experience of the operator performing the F.N.C. sampling, the diagnostic quality of the slides and the diagnostic accuracy rate [67].

4.3 Statistical analysis

The thyroid pathology pattern is described in terms of absolute Frequencies and Percentages. The 95% Confidence Intervals (CI) of percentages were calculated from the Normal distribution, except for small percentages where the exact probabilities from the binomial distribution were calculated [73]. The results obtained for BI and JHB were compared using the chi-square test, and its achieved power was determined using the obtained values of alpha, effect size and sample size (using G*Power) [74]. Differences between the relative frequencies of papillary cancer, follicular cancer and other thyroid neoplasias in JHB and BI were tested calculating the alpha and beta error of the chi-square test (G*Power). The 95%CI's of the papillary to follicular cancer ratios were calculated from the Normal distribution [73].

To evaluate the UIC, the mean value of three measurements done on each collected sample was determined. The median from these mean values was obtained as well as the 95% Confidence Interval (CI) of the Median (significance of the Kolmogorov-Smirnov test of normality < 0.000).

The iodine content of surface water is given as the Mean ± Standard Deviation of the Mean (significance of the Shapiro-Wilk test of normality = 0.945).

Most of the calculations were done in Excel [2007], using standard formulae. The normality tests were run in SPSS (version 21).

Chapter 5

Results

5.1 Cytology and Histology data from the Region of Beira Interior

A combined search was done of all patients that presented with thyroid nodular pathology at the local major referral Hospitals and, on a National level, at the Portuguese Oncology Institutes of Coimbra, Lisbon and Oporto that had a residential address originating from the designated area of Beira Interior. All cytology reports and all histology reports relating to the period dating from the start of January 2002 to the end of December 2007 were evaluated as to the type of pathology encountered and to the diagnosis.

A total of 869 patients were evaluated. From this total there were 607 Cytology reports and 278 Histology reports that were assessed, some patients having had both investigations.

The 607 cytology examinations were assessed as to their adequacy for enabling a cytodiagnosis. Cytology specimens that consisted mainly of blood and in which the thyroid follicular cellular material was considered scanty, absent, too degenerate or traumatized were considered unsuitable for cytodiagnosis and therefore classified as non diagnostic. Of the 607 cytology examinations a total of 74 were classified as being non diagnostic. In this retrospective analysis this represents a relatively high 12.19% non-diagnostic rate.

Some of these patients had a clinical indication for surgery. On the patients that were operated a definitive histological diagnosis was obtained. As a result, the number of non diagnostic cases in the evaluation of the final diagnosis group decreased to a total of 42. These patients were subsequently followed up clinically and whenever clinically indicated repeat cytological evaluation was performed.

Evaluation of the details of the above mentioned cytology and histology reports allowed the elaboration of a chart detailing the diagnosis of the 869 patients that presented with thyroid nodular pathology during the 6 year period between early January 2002 and the end of December 2007 in the iodine deficient area of Beira Interior of Portugal. These results are displayed in Figure 15.

Analysis of this data revealed that from the total of 869 patients, 143 of them had thyroid neoplasms. Looking at this group of 143 there were 31 patients with the diagnosis of papillary carcinoma and a total of 100 patients with follicular neoplasms. These included 10 follicular neoplasms diagnosed on cytological testing alone and therefore without the ability to detect the possibility of angio-invasion or capsular invasion, the diagnostic features of follicular carcinoma that can only be assessed on histology and not on isolated clusters of cells which as obtained through FNC. From this group of 100 there were also 18 patients with a diagnosis of follicular carcinoma and 17 patients with Hurthle cell lesions (13 diagnosed on cytology alone and 4 with a histological diagnosis of Hurthle cell adenoma).

The group of 143 patients with follicular neoplasms also included 4 patients with medullary thyroid carcinoma, 4 patients with anaplastic thyroid carcinoma and 1 patient with metastatic carcinoma into the thyroid.

The total of 869 patients that were evaluated also included 25 patients with a diagnosis of thyroiditis (12 of Hashimoto's, 5 of De Quervains, 3 acute supurative and 5 non-specific). There were also 298 benign colloid nodules and 245 multinodular goitres. There were 86 patients with benign thyroid cysts and 28 non-specific goitres. Rarer cases included 1 patient with a diagnosis of Grave's disease and 1 patient with a thyroglossal cyst.

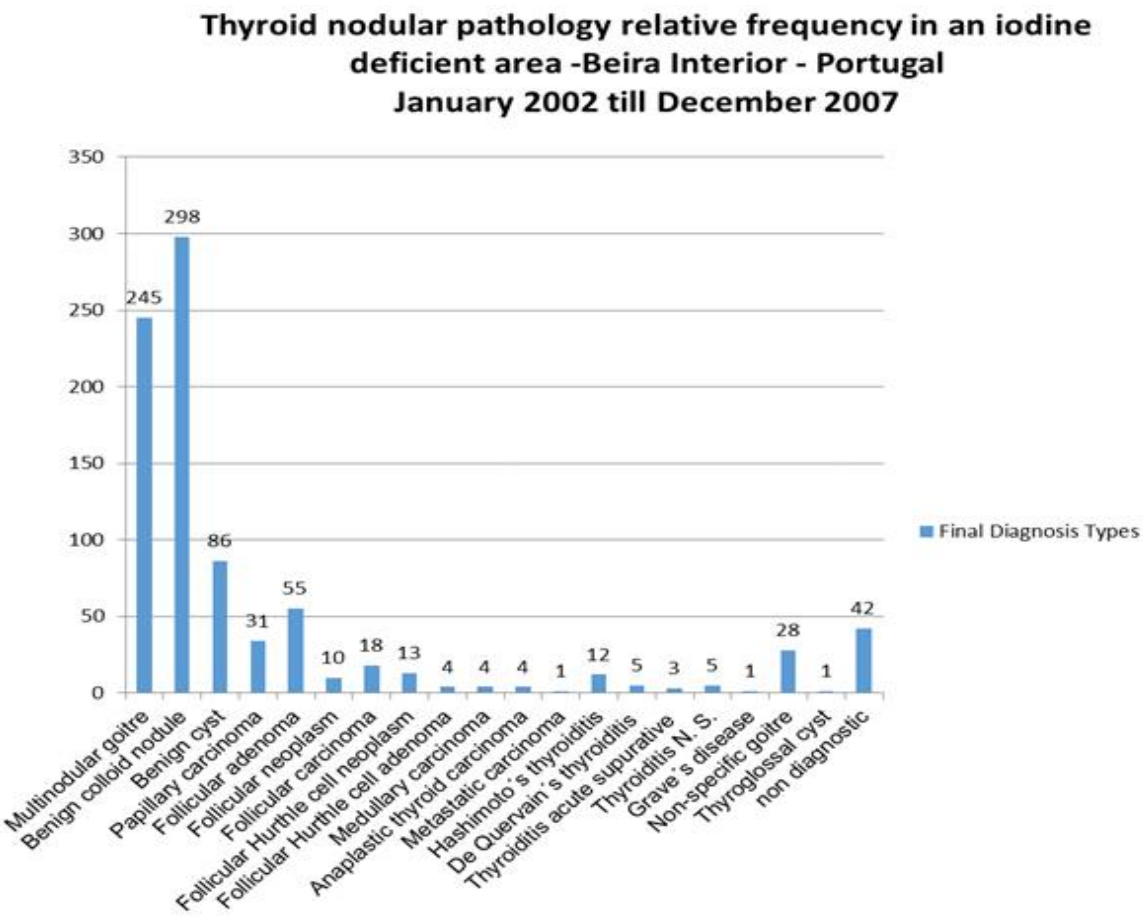


Figure 15. The relative frequencies of the different types of thyroid nodular pathology encountered in the population of the iodine deficient area of Beira Interior in Portugal for the 6 year period January 2002 to December 2007.

5.2 Histology results - Regions of Beira Interior and Johannesburg

The histology results from the region of BI *provided a total of* 278 patient´s histological exams *for evaluation (Figure 15). These were from 37 male patients and 241 female. Their ages ranged from 15 to 83 years of age (median 54 years).*

In the JHB area there was a total of *136 histological exams available (Figure16), 12 from male patients and 124 from female. Their ages ranged from 18 to 74 years (median 51 years).* The relative frequencies of the different pathology types in the two areas are displayed in table 1, which also displays an overlap in the 95%CI's of all histological types. The chi-square (two-sided) α error probability was 0.606 and an achieved power ($1 - \beta$) of 0.9987 was obtained in a post-hoc calculation. The papillary to follicular carcinoma ratios were close to 1 in both areas: BI area ratio: 1.4 with 95% CI from 0.816 to 2.434, and JHB area ratio: 0.87 with 95%CI from 0.412 to 1.821. The obtained chi-square (df=2) two-sided α error was 0.539 and the power of the test was 0.913 confirming the overlap shown in the 95% CI.

The analysis of the data from the Beira Interior region, in Portugal, revealed that 60 of the 278 patients had malignancies (21.2%). These were made up of 31 papillary carcinomas, 22 follicular cancers (18 follicular carcinoma and 4 *H*ürthle cell tumour), 3 medullary carcinomas and 4 anaplastic carcinomas (Figure 16). In the *JHB area 33 of the total of 136 patients (24.3%) had malignancies. These were made up of 13 papillary carcinomas, 15 follicular cancers (10 follicular carcinoma and 5 H*ürthle cell tumour), 1 medullary thyroid carcinoma, 3 anaplastic thyroid carcinomas and 1 metastatic carcinoma into the thyroid (Figure 17).

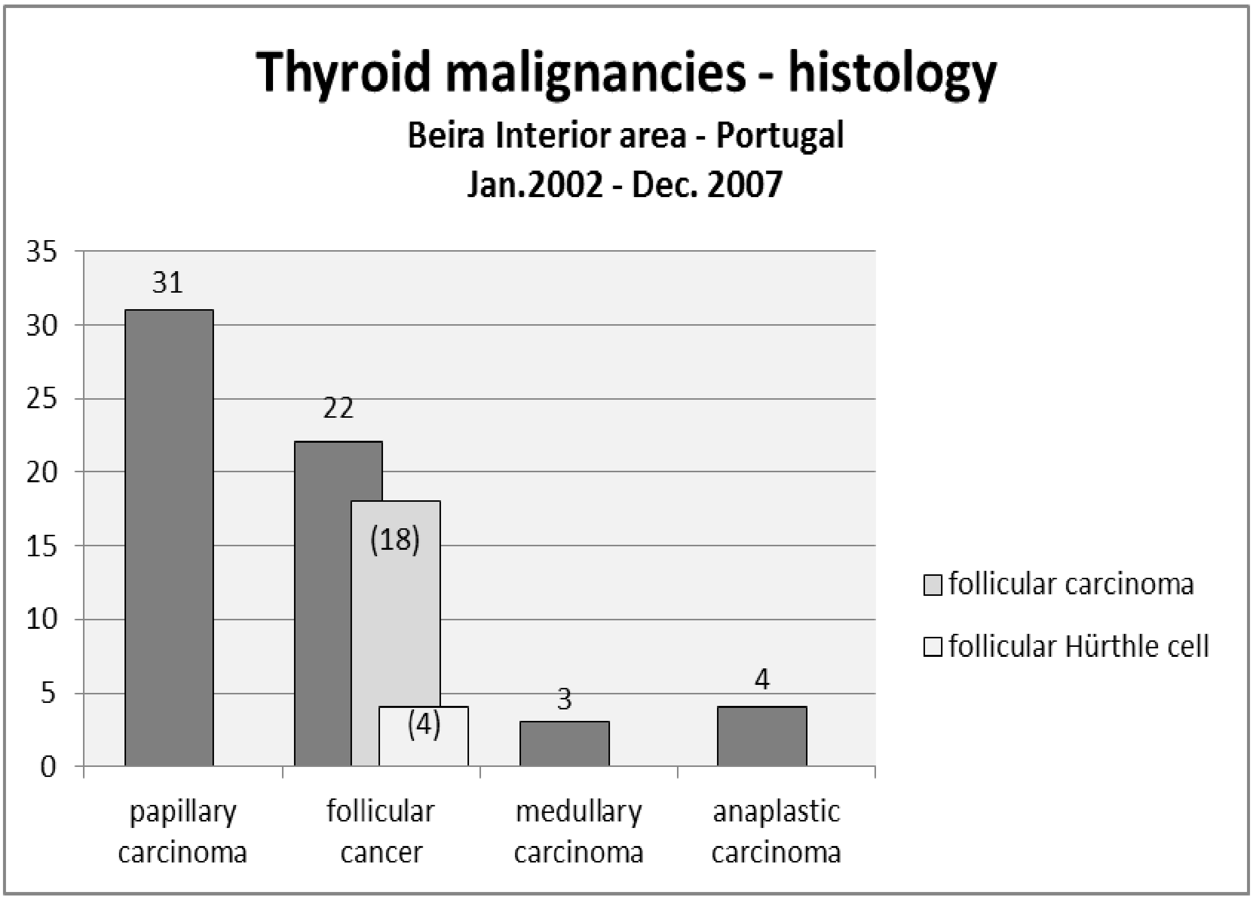


Fig. 16. Histological diagnosis: Thyroid malignancies – Beira Interior: 6 year period (January 2002-December 2007). The types of follicular cancer are discriminated.

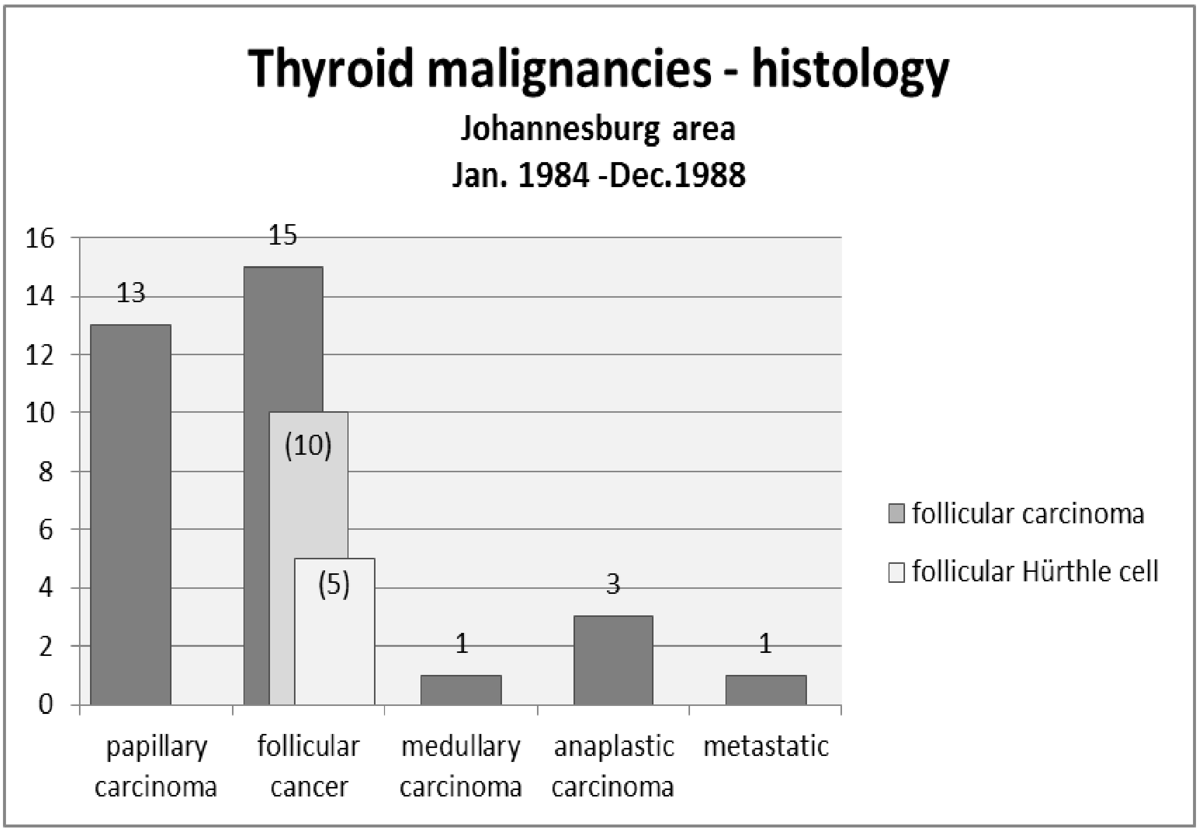


Fig. 17. Histological diagnosis: Thyroid malignancies – Johannesburg: 5 year period (January 1984- December 1988). The types of follicular cancer are discriminated

5.3 Combined evaluation of Iodine Nutrition - Beira Interior

*The results of the UIC measured from a general population sample of 214 volunteers by spot urine sampling showed a median UIC of 62.6*µg/L (95% CI: 57.5µg/L – 67.4µg/L). *Over a third of the samples (n=76/214) had UIC values below 50*µg/L *and* over 92% had UIC below 100 µg/L (n=197/214) (Figure 18).

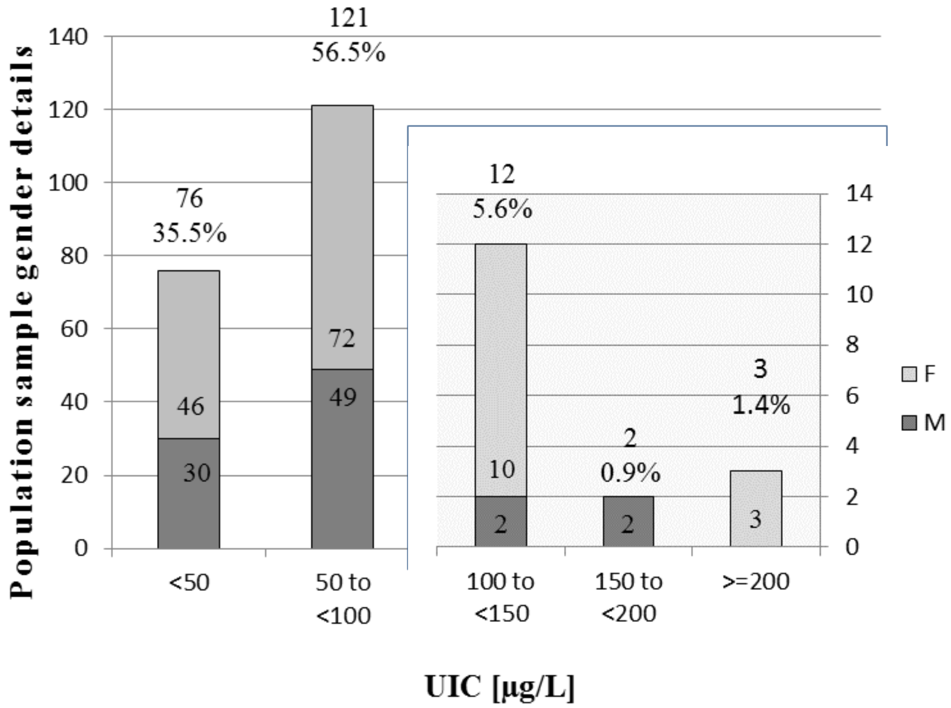


Fig. 18: Urinary Iodine Concentration (UIC) in population sample of 214 volunteers (131♀; 83♂) with ages ranging 8 to 97 years (median 52.0 years).

Measurements of the IC of the main surface water reservoir for BI (Penhas-da-Saúde) were done in a total of 13 samples from the middle of the reservoir and an equal number from the pre-treatment water side. The results of all samples were comparable and their mean value was 14.33µg/L ± 0.36µg/L.

*A total of 279 patients (*242 females and 37 males) *originating from the study area of BI region during the 6 year study period (January 2002 to December 2007) had thyroid surgery for thyroid nodular pathology. Their histology reports were assessed and used for the purpose of evaluating the relative incidence of the different types of thyroid nodular pathology in the study area.*

In the benign pathology group, there were 219 patients: 143 multinodular goitres, 15 benign colloid nodules, 50 follicular adenomas, 10 thyroiditis and 1 Graves' disease.

Analysing the thyroid neoplasms, there were in total 110, made up of 50 follicular adenomas (as mentioned above) and 60 histologic diagnoses of malignancies (21.5% of the total 279 patients).

These thyroid malignancies were: 31 papillary TC, 22 follicular TC (18 follicular carcinomas and 4 *H*ürthle cell tumours), 3 medullary thyroid cancers and 4 anaplastic TC (Figure 15).

The carcinomas of follicular cell origin (papillary, follicular and anaplastic) displayed a large female gender predominance of 82.5% and an overall female to male ratio of 4.7 (47/10).

In the medullary carcinomas (perifollicular or C cell origin) the opposite sex predominance was seen (0/3)although this disparity might be attributable to the relatively small numbers documented.

Except for the anaplastic TC, which had a mean age of 72.5 years, the ages for all the other cancer types reflect a fairly similar mean range in the 5th decade of life, ranging from the age of 26 to the age of 83 years (table 1).

The papillary to follicular (P:F) *carcinoma ratio was 31/22 = 1.41 (95% CI: 0.82 – 2.43).*

Chapter 6

Discussion

Iodine is broadly distributed in the environment in the form of iodide [1] and is concentrated in sea water and in marine life [5]. Under normal circumstances iodine is absorbed as iodide through the digestive tract mainly through the walls of the stomach and the small intestine [50], iodine deficiency usually resulting from inadequate iodine intake.

The recommended daily iodine intake is [23]: ≥120µg for schoolchildren (6-12 years); ≥150µg for adults; 250µg for pregnant or lactating women. Iodine deficiency occurs when the median UIC is below 100 µg/L [53,54].

From Portugal there is still no general population data on IN [43,45,66].

Regarding the iodine nutrition evaluation for the region of Beira Interior, in Portugal, the current study represents the first general population evaluation of IN in Portugal, from the inland region of BI. This was performed using the accepted population study method of the median UIC, through random spot urine samples [49]. Results showed a median UIC for the general population of BI of 62.6µg/L, with the added statistical validation of the 95% CI ranges that lie well below the reference value of 100µg/L.
Over 92% of the population sample had a low UIC of less than 100µg/L,
The similarity of the result for the region of BI in the current general population study, (median UIC 62.6µg/L) and the result reported for the region of BI of a median UIC 67.6µg/L in the only nationwide study on IN, that evaluated the population group of pregnant women´s IN [46] could, arguably, indicate that the results in this only national IN study from Portugal are likely to also reflect the general population IN levels. In support of this likely hypothesis, that the iodine intake is similar for the entire family, is the fact that, in Portugal, the majority of the population tend to have meals that are prepared for the entire family by a matriarch of the family. This likely hypothesis should, never the less, still be proven in a National general population study on IN, which hopefully will be spearheaded by the publication of the results from this present study.
Surface waters have been regarded as the best index of an environment´s iodine status, iodine in water representing the bioavailable form of the element [52]. The iodine content of the main surface water reservoir for BI, with mean value of 14.33µg/L. There is insufficient data available on iodine content of water reservoirs to enable establishing reference values.
The IC of water reservoirs is rarely measured or documented, this being the first documentation of the IC of this large main water reservoir of Penhas-da-Saúde, one of the largest surface water reservoirs in Portugal.
In Africa dietary ID is the major determinant of thyroid pathology, ID goitre being the most commonly observed thyroid disorder [75]. Substantiating evidence of the persistency of ID in

Africa has been the predominance of follicular carcinoma for decades [75], as opposed to usual papillary carcinoma predominance in iodine sufficient areas [9]. Recently, improvements in iodine intake led to reductions in goitre prevalence [75].

Baragwanath Hospital is the biggest Hospital in Africa and treats patients primarily from the JHB area. The 5 year histology evaluation in the JHB (BH) area in the present study (1984-1988), relates to a time when iodine deficiency was present in this region, before iodine supplementation legislation requiring all table salt manufactured in South Africa to contain potassium iodate was introduced in 1995 [47].

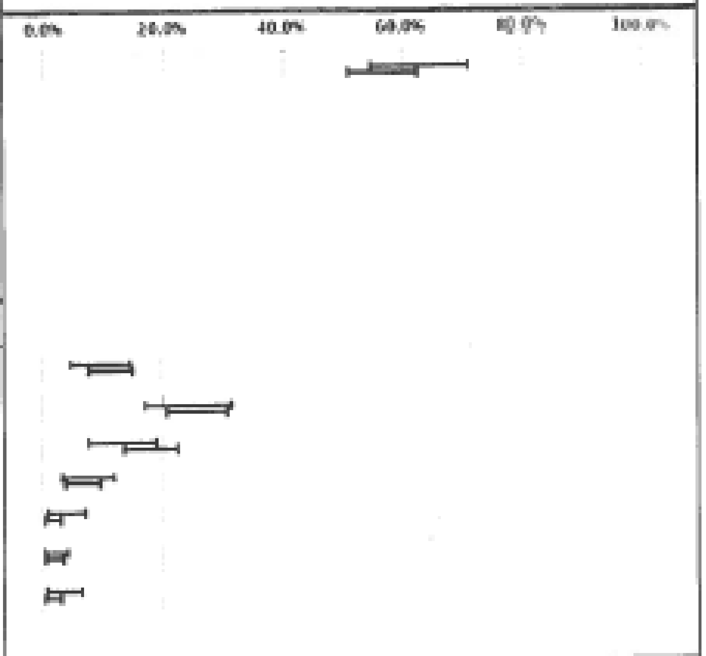
Evaluating the thyroid pathology data from both study areas (displayed in Table 1), the results showed coinciding values with regard to the relative incidences of the different histology types demonstrated by the overlapping 95% CI´s of the each thyroid neoplasia. This similarity of the thyroid malignancies patterns was also found using the achieved power of the chi-square test. The malignancy rate in the inland region of BI, in Portugal was 21.2% (60 malignancies from a total of 278 patients) and in the JHB(BH) area during the 5 year evaluated period was 24.3% (33 malignancies out of a total of 136 patients that undergone thyroid surgery). The histology pattern displayed in both study areas was indicative of ID [9], particularly regarding the papillary to follicular carcinoma ratios, that were close to 1 in both areas, and the relatively high anaplastic thyroid carcinoma incidences: BI area papillary/follicular ratio of 1.4 (95% CI: 0.816 to 2.434), with a total of 4 anaplastic carcinomas out of a total of 60 malignancies (6.7%); JHB area papillary/follicular ratio of 0.87 (95% CI: 0.412 to 1.821) and a total of 3 anaplastic carcinomas out of a total of 33 malignancies (9.1%) . The malignancy rates reflect the selection criteria for surgery based on cytology results, as well as the existing ID in both areas in the study period.

Table 1 - Frequency of different pathology types in the two study areas

	Johannesburg South Africa 5 years period N=136			Beira Interior Portugal 6 years period N=278		
	n(n/N)	95% CI		n(n/N)	95% CI	
benign:	85(62.5%)	54.4%	70.6%	168(60.4%)	54.9%	66.2%
benign colloid nodules	85(62.5%)	54.4%	70.6%	157(56.5%)	50.7%	62.3%
Grave's disease	-	-	-	1(0.4%)	0.01%	1.29%
thyroiditis:	-	-	-	10(3.6%)	1.4%	5.8%
Hashimoto's	-	-	-	6(2.2%)	0.5%	3.9%
non-specific	-	-	-	3(1.1%)	0.22%*	3.07%*
acute supparative	-	-	-	1(0.4%)	0.01%	1.29%
neoplastic:	51(37.5%)	29.4%	45.7%	110(39.6%)	33.8%	45.3%
papillary carcinoma	13(9.6%)	4.6%	14.5%	31(11.2%)	7.5%	14.9%
follicular neoplasm:	33(24.3%)	17.1%	31.5%	72(25.9%)	20.8%	31.1%
follicular adenoma	18(13.2%)	7.5%	19.0%	50(18.0%)	13.5%	22.5%
follicular carcinoma	10(7.4%)	3.0%	11.7%	18(6.5%)	3.6%	9.4%
folli.Hürthle cell tumour	5(3.7%)	0.5%	6.9%	4(1.4%)	0.39%*	2.56%*
medullary th. carcinoma	1(0.7%)	0.02%*	3.95%*	3(1.1%)	0.22%*	3.07%*
anaplastic th. carcinoma	3(2.2%)	0.46%*	6.22%*	4(1.4%)	0.39%*	2.56%*
metastatic carcinoma	1(0.7%)	0.02%*	3.95%*			

graphic display of 95% CI

(upper, grey bar: Jhb – lower, black bar: B.I.)



* calculated using the exact probabilities of the Binomial distribution (np<5)

Statistics relating to the economically depressed study region of BI are probably underrepresented, considering that approximately 30% of the population in this area are over the age of 65 years, (60,260 of a population of 211,205) [71] whose offspring tend to migrate to the more affluent coastal metropolitan areas of the country, where they work and live. Should these elderly patients require surgery, particularly if the possibility of cancer is mentioned, they would tend to have it in the metropolitan residential areas of their younger relatives, being statistically lost from their usual area of residence in BI.

Voluntary salt iodization at a low iodine concentration was not successful in eliminating ID in SA before the introduction of mandatory salt iodization at a higher iodine concentration in 1995 [47]. This measure has succeeded in the elimination of ID throughout South Africa [48].

China successfully eliminated ID using a coordinated approach which started in 1978 with the ruling that the cost of iodization of salt be borne by the state, as this would be to the benefit of the entire country´s population [76].

In Portugal, where the existing legislation relating to salt iodization was adopted in 1969 on a voluntary basis [77], there is still a lack of national general population data regarding INu [77,78], no national committee existing for monitoring the elimination of ID [77]. This voluntary salt iodization legislation has been shown ineffective in the only countrywide published study on INu, done in the selected population group of Portuguese pregnant women, which demonstrated inadequate iodine intake throughout Portugal [46], with a nationwide median UIC value was 82.5µg/L. The region of BI was the most iodine deficient area of Continental Portugal with a median UIC of 67.6µg/L, illustrating that regarding INu it´s the amount of iodine ingested that matters and not necessarily the proximity to the sea, the mid Atlantic islands of Açores had an even lower median UIC of 50.0µg/L [46]. Two additional, complementary studies on INu in Portugal were recently published, (in Portuguese, English Abstract only), done on schoolchildren aged 6-12, from 78 schools from the region of Continental European Portugal [79] and from the regions of the Atlantic Islands of Madeira and Açores (school children and pregnant women aged 17-46 years) [80], displaying results that could be considered in keeping with the only nationwide study available, previously cited [46].

These three recent studies [46,79,80] and the influence exerted by the working group on Thyroid Studies of the Portuguese Endocrine Society, led to the publication of “Guidelines” in August 2013 [81], by the Directorate General of Health, recommending the prescription of a daily supplement of potassium iodide (KI) in a dose of 150 to 200µg to pregnant, breastfeeding or pre-pregnancy age women. This value is inferior to the recommended amount by the WHO of 250µg daily [53]. Whilst these developments are positive in attempting to eliminate ID, the issuing of guidelines, without supporting legislation or the combination of other complementary measures (adequate public information regarding the consequences of ID) may well fail to produce the expected results. The effectiveness of these guidelines still has to be proven.

The successful correction of ID requires easy and affordable access to iodized salt by the general population. The implementation in China, in 1978, of the policy that the cost of salt iodization should be borne by the Government, combined with adequate transportation, packaging and distribution measures ensured the availability and quality control throughout the vast country. This contributed to the success in the elimination of ID [76,82]. These measures recognised the benefits to the entire population and to the country of access to iodized salt. China's successful strategies in the elimination of IDD were achieved through the combination of carefully implemented salt iodization programs combined with on-going sub-national population monitoring for iodine nutrition [82]. It´s success made it an example to be followed.

In South Africa the price of iodized salt is the same as for non iodized salt and both have been generally available countrywide even before the introduction of mandatory salt iodisation at a higher level than before, in 1995. By contrast, in Portugal iodized salt (with specification of iodine content) is generally not available. The only brand available is priced at more than double that of non-iodized salt (unpublished results). Since Portugal has salt production facilities [77] legislation requiring all commercially available salt to have a set amount of potassium iodide, as was done in South Africa in 1995 [47], could easily be implemented. To achieve the aim of eliminating ID adequate public information highlighting the serious adverse effects of iodine deficiency could provide the motivation for people to adopt a pro-active stand regarding the use iodized salt. Additionally promoting the consumption of sea generated food products, naturally iodine containing, would be an important complementary measure, especially at times when reducing daily salt intake is now generally recommended.

Chapter 7

Publications in support of this Thesis: Published Articles and Abstracts. Presentations.

Published Articles:

1) Santos JEC et al BMC Research Notes DOI 10.1186_s13104_015_1155-3_2015_1155_OnlinePDF

Santos JEC, Kalk WJ, Freitas M, Marques Carreira I, Castelo Branco M. Iodine deficiency and thyroid nodular pathology – epidemiological and cancer characteristics in different populations: Portugal and South Africa. BMC Research Notes 2015; 8:284-291.

Santos *et al. BMC Research Notes* (2015) 8:284
DOI 10.1186/s13104-015-1155-3

BMC Research Notes

RESEARCH ARTICLE Open Access

Iodine deficiency and thyroid nodular pathology - epidemiological and cancer characteristics in different populations: Portugal and South Africa

José Eduardo Carvalho Santos[1,2,3*], William John Kalk[4], Miguel Freitas[3,5], Isabel Marques Carreira[6] and Miguel Castelo Branco[1,2,3]

Abstract

Background: The prevalence and pathology pattern of iodine deficiency (ID) related disorders are influenced by the dietary iodine intake: low iodine leads to thyroid nodular enlargement, to an increase in the incidence of thyroid cancer, an increase in anaplastic carcinomas and to an alteration in the papillary to follicular neoplasia ratio. This study aims at highlighting the effects of ID by comparatively evaluating the pattern of thyroid nodular pathology in different populations that, although geographically distant and heterogeneous, both had iodine deficiency at the time of data gathering and are at high altitude: Beira Interior (BI) in Portugal and Johannesburg (JHB) in South Africa. (S.A.) Mandatory salt iodization introduced in S. A. in 1995 has recently been shown to have resulted in the correction of ID.

Methods: Evaluation of thyroid histology reports over a 6 year period in BI and a 5 year period in the JHB area.

Results: Region of BI: 278 patients with histology reports-60 were malignancies (21.2 %): 31 papillary carcinomas, 22 follicular cancers (18 follicular carcinomas and 4 Hürthle cell tumours), 3 medullary carcinomas and 4 anaplastic carcinomas. Region of JHB: 136 histology reports- 33 were malignancies (24.3 %): 13 papillary carcinomas, 15 follicular cancers (10 follicular carcinomas and 5 Hürthle cell tumours), 1 medullary carcinoma, 3 anaplastic carcinomas and 1 metastatic carcinoma into the thyroid. There was an overlap in the frequencies of all histology types, of particular relevance in the relatively high anaplastic carcinoma incidences and in the papillary to follicular carcinoma ratios which was close to 1 in both areas- BI area ratio: 1.4 and JHB area ratio: 0.87, with overlapping 95 % CI's, also confirmed by the results of the chi-square calculations.

Conclusions: During the study periods evaluated both study areas displayed pathology patterns usually found in ID. Public information regarding the negative consequences of ID combined with the availability of affordable iodized salt are likely to achieve the goal of the elimination of ID. Sea based nutrition, (naturally iodine containing), may also contribute to the elimination of ID, particularly at times when salt restriction tends to be generally advised.

Keywords: Thyroid, Goiter, Papillary carcinoma, Follicular carcinoma, Anaplastic carcinoma, Iodine deficiency, Iodine nutrition, Iodized salt, Seafood nutrition

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Background

Iodine is the essential component of the thyroid hormones T3 and T4 [1], which regulate metabolic processes in most cells and play an important role in the early growth and development of most organs, particularly the brain [2]. Globally iodine deficiency (ID) is the most common preventable cause of brain damage, with more than 2 billion people from 130 countries at risk [3], including an estimated 241 million children of school age [4]. It is especially prevalent worldwide in inland continental or mountainous regions [1], in areas beyond high mountain ranges [5] and may be independent of sea proximity. Consequences of ID include variable degrees of intellectual impairment, with demonstrable neuropsychointellectual deficits [6, 7], compromised reproductive potential [8], development of goitre, thyroid nodular pathology [1] and an increase in the incidence of thyroid cancer [9, 10]. Low iodine intake leads to an adaptive process that results in sustained increased secretion of thyroid-stimulating hormone (TSH), which has a direct trophic effect on the thyroid gland leading to hypertrophy and hyperplasia [1] that progresses to nodular development [1], focal hyperplasia, adenoma formation, autonomous growth and even malignant transformation [11]. ID has been shown to be associated not only with an increase in thyroid cancer incidence rate but also with an alteration in the ratio of papillary to follicular (P:F) neoplasia, from the usual significant predominance of papillary carcinoma observed in iodine sufficiency [9]. The P:F thyroid cancer ratio is reported as ranging from 6.5:1 to 3.4:1 in areas of high iodine intake, and 1.7:1 to 0.19:1 in ID areas [12]. This ID pattern has been shown to be reversed when iodine deficiency is corrected [9].

South Africa is documented as having had areas of ID in the years before the introduction in 1995 of legislation requiring all manufactured salt to contain potassium iodate [13]. This policy has been shown successful in the elimination of ID in South Africa [14]. In Portugal salt iodisation is done on a voluntary basis, there being no national program for the elimination of ID and no regular Urinary Iodine (UI) monitoring [15]. There has been no general population data on iodine nutrition (INu) [4, 16] the only countrywide study available referring to the population group of pregnant women, demonstrating significant iodine deficiency throughout the country [17].

In population studies, measuring the median urinary iodine concentration (UIC) is the most accurate method of assessing and monitoring the INu status, through random spot urine samples measured in μg/L [18]. Iodine deficiency has been successfully corrected in S.A. [14] following the introduction of mandatory salt iodization in 1995 [13]. In the years preceding that, evaluation of INu using UIC was not easily nor routinely available. An alternative method of assessing INu through parameters relating to the thyroid histology (THist) pattern has been previously referred to and documented [9].

The aim of the present study is to evaluate the thyroid histology pattern of the population of the inland region of BI in Portugal and to compare it with the available histology pattern from the population of JHB, South Africa, prior to mandatory iodization of salt. These areas had in common: ID during the time periods evaluated [13, 17] and being high altitude regions, away from the coast. The consequences of inadequate iodine intake, the importance of adequate public information on this issue, salt iodization and of the need for consumption of sea food based nutrition are highlighted.

Methods

Study population

This study was approved by the ethics committees of the Faculty of Health Sciences of the University of Beira Interior and of Centro Hospitalar Cova da Beira (Hospitals of Covilhã and Fundão), as well as by the Committee for Research on Human Subjects of the University of the Witwatersrand/Baragwanath Academic Hospital.

The ultimate aim of the current study is to contribute towards the elimination of ID in countries and regions in which this problem is still prevalent, highlighting it's serious negative consequences and proposing possible solutions that are easily applicable and affordable. Towards this aim, a comparative evaluation of the THist patterns from the population of the inland region of BI in Portugal and from the population from JHB, reflected by the predominantly black African population seen at the largest referral Hospital in S. A., Baragwanath Hospital (BH) was sought. The available thyroid histopathology pattern from the JHB, S.A. area, relating to a period prior to the introduction of mandatory salt iodization in 1995, was used [19].

The study area of BI, in Portugal is situated beyond the mountain range of Serra da Estrela, a mountainous range stretching across 115 km, with its highest peak at 1993 m above sea level [20]. The area of JHB is situated in a plateau at approximately 1750 m of altitude [21].

The introduction of mandatory salt iodization in South Africa [13] has been proven successful in eliminating ID [14]. Whilst this noteworthy success demonstrates an easy to follow example for regions that still experience the problem of ID it also means that UIC data demonstrating the previous ID status in South Africa would have had to be obtained from publications that preceded this measure. The evaluation of the histopathology pattern and its characteristics is an alternative and complementary way to measurements of UIC in demonstrating ID, as has been highlighted in previous studies [9].

Diagnosis evaluation by histology

Patients that had histology available had been selected for thyroid surgery, in both areas, based on cytology criteria, ultrasound findings or clinical indications. These included family history of thyroid malignancy, an enlarging nodule or the presence of neck lymphadenopathy. A representative and illustrative sample of the thyroid nodular pathology in each of these areas was sought so as to enable a comparative evaluation of the patterns of pathology in these two areas.

Histology reports relating to patients residing in the inland region of BI, performed during the period between January 2002 and December 2008 were evaluated. The 6 year time period was arbitrarily chosen, aiming at obtaining a representative sample of the thyroid nodular pathology in the area. All reports from the local major referral Hospitals linked to the University of Beira Interior, (the only State University in this region), namely the Hospitals of Covilhã and Fundão, were obtained. This data was complemented with histology reports from the Portuguese Oncology Institutes of the three main nationwide centres of Lisbon, Oporto and Coimbra (the latter being the main Oncology referral centre for the region of BI), from patients that provided a residential address from the study area of BI.

Thyroid histology reports evaluated from the JHB area related to patients that had presented to the BH thyroid clinic in JHB during the 5 year period between January 1984 and December 1988 [19], this corresponding to a time preceding the introduction of mandatory salt iodation in South Africa [13].

The standard method for general histology with hematoxylin and eosin staining (H&E) was employed in both study areas, histology examinations having been performed by specialist pathologists with an interest in thyroid pathology at Academic Pathology Centres. The diagnostic criteria followed at both centres: CEDAP, linked to the University of Coimbra and at the South African Institute for Medical Research (S.A.I.M.R.), in Johannesburg, connected to the University of the Witwatersrand, followed the same diagnostic criteria, as defined by the W.H.O.

Although the time periods for data collection were different, they both correspond to periods when ID prevailed in each study area [13, 17].

Statistical analysis

The thyroid pathology pattern is described in terms of absolute Frequencies and Percentages. The 95 % Confidence Intervals (CI) of percentages were calculated from the Normal distribution, except for small percentages where the exact probabilities from the binomial distribution were calculated [22]. The results obtained for BI and JHB were compared using the chi-square test, and its achieved power was determined using the obtained values of alpha, effect size and sample size (using G*Power) [23]. Differences between the relative frequencies of papillary cancer, follicular cancer and other thyroid neoplasias in JHB and BI were tested calculating the alpha and beta error of the chi-square test (G*Power). The 95 %CI's of the papillary to follicular cancer ratios were calculated from the Normal distribution [22].

Results

The histology results from the region of BI provided a total of 278 patient's histological exams for evaluation. These were from 37 male patients and 241 female. Their ages ranged from 15 to 83 years of age (median 54 years). In the JHB area there was a total of 136 histological exams available, 12 from male patients and 124 from female. Their ages ranged from 18 to 74 years (median 51 years) [19]. The relative frequencies of the different pathology types in the two areas are displayed in Fig. 1, which also displays an overlap in the 95 %CI's of all histological types. The chi-square (two-sided) α error probability was 0.606 and an achieved power $(1 - \beta)$ of 0.9987 was obtained in a post-hoc calculation. The papillary to follicular carcinoma ratios were close to 1 in both areas: BI area ratio: 1.4 with 95 % CI from 0.816 to 2.434, and JHB area ratio: 0.87 with 95 %CI from 0.412 to 1.821. The obtained chi-square $(df = 2)$ two-sided α error was 0.539 and the power of the test was 0.913 confirming the overlap shown in the 95 % CI.

The analysis of the data from the Beira Interior region, in Portugal, revealed that 60 of the 278 patients had malignancies (21.2 %). These were made up of 31 papillary carcinomas, 22 follicular cancers (18 follicular carcinoma and 4 Hürthle cell tumour), 3 medullary carcinomas and 4 anaplastic carcinomas (Fig. 2). In the JHB area 33 of the total of 136 patients (24.3 %) had malignancies. These were made up of 13 papillary carcinomas, 15 follicular cancers (10 follicular carcinoma and 5 Hürthle cell tumour), 1 medullary thyroid carcinoma, 3 anaplastic thyroid carcinomas and 1 metastatic carcinoma into the thyroid (Fig. 3).

Discussion

Iodine is broadly distributed in the environment in the form of iodide [1] and is concentrated in sea water and in marine life [5]. Under normal circumstances iodine is absorbed as iodide through the digestive tract mainly through the walls of the stomach and the small intestine [24], iodine deficiency usually resulting from inadequate iodine intake.

	Johannesburg South Africa 5 years period N=136			Beira Interior Portugal 6 years period N=278		
	n(n/N)	95% CI		n(n/N)	95% CI	
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benign colloid nodules	85(62.5%)	54.4%	70.6%	157(56.5%)	50.7%	62.3%
Grave's disease	-	-	-	1(0.4%)	0.01%	1.29%
thyroiditis:	-	-	-	10(3.6%)	1.4%	5.8%
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non-specific	-	-	-	3(1.1%)	0.22%*	3.07%*
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follicular adenoma	18(13.2%)	7.5%	19.0%	50(18.0%)	13.5%	22.5%
follicular carcinoma	10(7.4%)	3.0%	11.7%	18(6.5%)	3.6%	9.4%
folli.Hürthle cell tumour	5(3.7%)	0.5%	6.9%	4(1.4%)	0.39%*	2.56%*
medullary th. carcinoma	1(0.7%)	0.02%*	3.95%*	3(1.1%)	0.22%*	3.07%*
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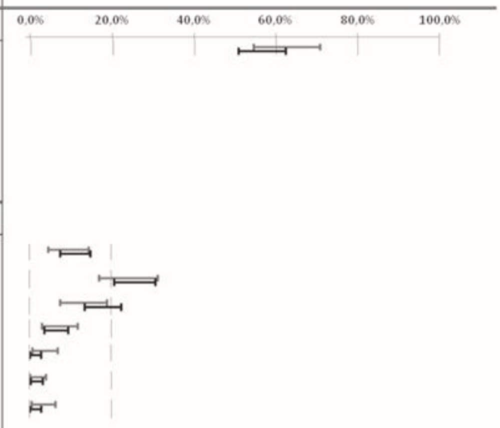

Fig. 1 Frequency of different pathology types in the two study areas. *calculated using the exact probabilities of the Binomial distribution (np<5)

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Baragwanath Hospital is the biggest Hospital in Africa and treats patients primarily from the JHB area. The 5 year histology evaluation in the JHB (BH) area in the present study (1984–1988), relates to a time when iodine deficiency was present in this region, before iodine supplementation legislation requiring all table salt manufactured in South Africa to contain potassium iodate was introduced in 1995 [13].

Evaluating the thyroid pathology data from both study areas (displayed in Fig. 1), showed coinciding values in the relative incidences of the different histology types as demonstrated by the overlapping 95 % CI's of each thyroid neoplasia. This similarity of the thyroid malignancy patterns was also confirmed by the achieved power of the chi-square test.

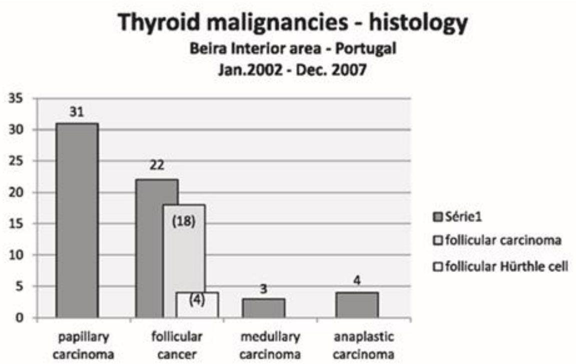

Fig. 2 Histological diagnosis (illustrative display)—Thyroid malignancies—Beira Interior: 6 year period (January 2002–December 2007). The types of follicular cancer are discriminated

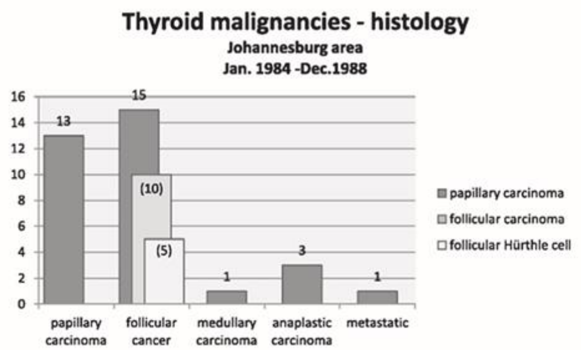

Fig. 3 Histological diagnosis (illustrative display)—Thyroid malignancies—Johannesburg: 5 year period (January 1984–December 1988). The types of follicular cancer are discriminated

The malignancy rate in the inland region of BI, in Portugal was 21.2 % (60 malignancies from a total of 278 patients) and in the JHB (BH) area during the 5 year evaluated period was 24.3 % (33 malignancies out of a total of 136 patients that undergone thyroid surgery).

The histology pattern displayed in both study areas was indicative of ID [9], particularly regarding the papillary to follicular carcinoma ratios, that were close to 1 in both areas, and the relatively high anaplastic thyroid carcinoma incidences: BI area papillary/follicular ratio of 1.4 (95 % CI: 0.816 to 2.434), with a total of 4 anaplastic carcinomas out of a total of 60 malignancies (6.7 %); JHB area papillary/follicular ratio of 0.87 (95 % CI: 0.412 to 1.821) and a total of 3 anaplastic carcinomas out of a total of 33 malignancies (9.1 %). The malignancy rates reflect the selection criteria for surgery based on cytology results, as well as the existing ID in both areas in the study period.

Statistics relating to the economically depressed study region of BI are probably underrepresented, considering that approximately 30 % of the population in this area are over the age of 65 years, (60,260 of a population of 211,205) [26] whose offspring tend to migrate to the more affluent coastal metropolitan areas of the country, where they work and live. Should these elderly patients require surgery, particularly if the possibility of cancer is mentioned, they would tend to have it in the metropolitan residential areas of their younger relatives, being statistically lost from their usual area of residence in BI.

Voluntary salt iodization at a low iodine concentration was not successful in eliminating ID in SA before the introduction of mandatory salt iodization at a higher iodine concentration in 1995 [13]. This measure has succeeded in the elimination of ID throughout South Africa [14].

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In Portugal, where the existing legislation relating to salt iodization was adopted in 1969 on a voluntary basis [15], there is still a lack of national general population data regarding INu [15, 16], no national committee existing for monitoring the elimination of ID [15]. This voluntary salt iodization legislation has been shown ineffective in the only countrywide published study on INu, done in the selected population group of Portuguese pregnant women, which demonstrated inadequate iodine intake throughout Portugal [17], with a nationwide median UIC value was 82 · 5 μg/L. The region of BI was the most iodine deficient area of Continental Portugal with a median UIC of 67 · 6 μg/L. Illustrating that INu is determined by the amount of iodine ingested and not necessarily the proximity to the sea, the mid Atlantic islands of Açores had an even lower median UIC of 50 · 0 μg/L [17]. Two additional, complementary studies on INu in Portugal were recently published, (in Portuguese, English Abstract only), done on schoolchildren aged 6–12, from 78 schools from the region of Continental European Portugal [28] and from the regions of the Atlantic Islands of Madeira and Açores (school children and pregnant women aged 17–46 years) [29], displaying results that could be considered in keeping with the only nationwide study available, previously cited [17]. These three recent studies [17, 28, 29] and the influence exerted by the working group on Thyroid Studies of the Portuguese Endocrine Society, led to the publication of "Guidelines" in August 2013 [30], by the Directorate General of Health, recommending the prescription of a daily supplement of potassium iodide in a dose of 150 to 200 μg to pregnant, breastfeeding or pre-pregnancy age women. Whilst these developments are positive in attempting to eliminate ID, the issuing of guidelines, without supporting legislation or the combination of other complementary measures (adequate public information regarding the consequences of ID) may well fail to produce the expected results. The effectiveness of these guidelines still has to be proven.

The successful correction of ID requires easy and affordable access to iodized salt by the general population. The implementation in China, in 1978, of the policy that the cost of salt iodization should be borne by the Government, combined with adequate transportation, packaging and distribution measures ensured the availability and quality control throughout the vast country. This contributed to the success in the elimination of ID [27, 31]. These measures recognized the benefits to the entire population and to the country of access to iodized salt. China's successful strategies in the elimination of IDD were achieved through the combination of carefully implemented salt iodization programs combined with ongoing sub-national population monitoring for iodine nutrition [31]. Its success made it an example to be followed.

In South Africa the price of iodized salt is the same as for non iodized salt and both have been generally available countrywide, even before the introduction in 1995 of mandatory salt iodization at a higher level than before. By contrast, in Portugal iodized salt (with specification of iodine content) is generally not available. The only brand available is priced at more than double that of non-iodized salt (unpublished results). Since Portugal has salt production facilities [15] legislation requiring all commercially available salt to have a set amount of potassium iodide, as was done in South Africa in 1995 [13], could easily be implemented. To achieve the aim of eliminating ID adequate public information highlighting the serious adverse effects of iodine deficiency could provide the motivation for people to adopt a pro-active stand regarding the use iodized salt. Additionally, promoting the consumption of sea generated food products, naturally iodine containing, would be an important complementary measure, especially at times when reducing daily salt intake is now generally recommended.

Conclusions

The study regions evaluated of BI in Portugal and JHB in South Africa display patterns of thyroid nodular pathology that were shown to be similar in both study populations, during the periods evaluated, indicative of ID as evidenced particularly by the papillary to follicular carcinoma ratios (close to 1 in both study areas) and the relatively high number of anaplastic carcinomas [9, 12]. This in spite of the differences in geographical location, the racial and ethnic composition of the population samples, as well as the different time periods of evaluation.

The example of South Africa's success in changing from having ID in the past to its current iodine sufficiency status shows that the elimination of ID is achievable provided there is easy and affordable access to iodized salt and especially if the population is adequately informed as to the serious negative consequences of iodine deficiency. Promoting sea based nutrition could also be important.

These measures could be applicable, as public health measures, to other populations in different parts of the world still experiencing the problem of ID.

Abbreviations

BH: Baragwanath hospital; BI: Beira interior; CI: Confidence intervals; H&E: Hematoxilin and eosine; ID: Iodine deficiency; IDD: Iodine deficiency disorders; INu: Iodine nutrition; JHB: Johannesburg; SA: South Africa; TSH: Thyroid stimulating hormone; T3: Tri-iodothyronine; T4: Thyroxine; THist: Thyroid histology; UIC: Urinary iodine concentration; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JECS contributed to the conception and design of the study, the major writing of the manuscript as well as the acquisition of data in JHB, South Africa; and the majority of the data from BI, Portugal; he coordinated the statistical design and the data interpretation. WJK participated in the sequence alignment critical revision and correction of the manuscript. MF contributed with the elaboration and writing of the statistical analysis, as well as with the elaboration and design of the figures. IMC contributed to the sequence alignment, critical revision and correction of the manuscript. MCB participated in the overall supervision and critical evaluation of the study, as well as the critical revision and correction of the study. All authors read and approved the final manuscript.

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Iodine deficiency a persisting problem: assessment of iodine nutrition and evaluation of thyroid nodular pathology in Portugal

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ORIGINAL ARTICLE

Iodine deficiency a persisting problem: assessment of iodine nutrition and evaluation of thyroid nodular pathology in Portugal

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Abstract

Background The goal of eliminating iodine deficiency (ID) by the year 2000 has still not been achieved in several countries. More than 2 billion people worldwide (over 260 million school age children) remain ID. In Europe, there are still countries, such as Portugal, without national general population data on iodine nutrition (IN). This study aims at evaluating combined complementary data of the IN of the general population through urinary iodine concentration (UIC) and the thyroid histology profile from the inland region of Beira Interior (BI), in Portugal.

Methods UIC from a population sample of 214 volunteers (131 females and 83 males), with ages ranging from 8 to 97 years (mean 51.5 years ± SD 20.74 years), from BI was determined; the thyroid histology pattern in BI (6-year period) was evaluated; and the iodine content of the largest surface water reservoir of BI, never previously reported, was measured.

Results Median UIC of 62.6 µg/L was measured. Over 92 % of the population had UIC less than 100 µg/L. From 279 histology reports evaluated, the incidence of the different types of thyroid nodular pathology in BI was established. There were 60 histologic diagnoses of malignancy. The observed ratio of papillary to follicular carcinoma relatively close to 1 and the fairly high percentage of anaplastic carcinomas are characteristic of ID areas.

Conclusions The findings of this first general population study on IN from the inland region of BI, Portugal, document significant ID. This problem, with its serious public health implications, could be corrected by having affordable iodised salt widely and generally available and by promoting a proactive population attitude generated by ample public information and educational programs as to the negative consequences of ID.

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Keywords Iodine deficiency · Thyroid nodular pathology · Thyroid cancer · Papillary thyroid cancer · Follicular thyroid cancer · Anaplastic thyroid cancer · Urinary iodine concentration · Seafood nutrition · Iodised salt

Abbreviations

ATC	Anaplastic thyroid cancer
BI	Beira Interior
CI	Confidence interval
H&E	Hematoxilin and eosine
FTC	Follicular thyroid cancer
IC	Iodine content
ID	Iodine deficiency
IDD	Iodine deficiency disorders
IN	Iodine nutrition
PF	Papillary to follicular
PTC	Papillary thyroid cancer
TC	Thyroid cancer
UIC	Urinary iodine concentration
UK	United Kingdom
USA	United States of America

Introduction

Twenty-five years have elapsed since all attending countries to the World Summit for Children at the United Nations, New York, in 1990, endorsed the goal of the virtual elimination of ID by the year 2000 [1], ID having been considered a major public health problem [2]. This recognised the importance of ID as a cause of brain damage [1] and the most common cause of preventable mental impairment worldwide [3, 4]. Over two billion people worldwide have insufficient iodine intake, including an estimated 266 million children of school age [5].

The prevalence and pathology patterns of iodine deficiency disorders (IDD) reflect the population's dietary intake [6]. This may be influenced by culture, difficulties in accessing iodine containing foods or products.

Papillary thyroid cancer (PTC) is the predominant form of thyroid cancer (TC) where iodine sufficiency exists [7, 8], with papillary-to-follicular (P/F) ratios of 3.4–6.0:1 reported from the USA [8]. By contrast, in iodine-deficient areas follicular thyroid cancer (FTC) tends to predominate [7–9]. Geographical areas where ID prevails therefore display an inversion in the usual frequency ratio of P/F thyroid cancer. They also have a relative increase in the incidence of anaplastic carcinomas [9, 10].

Iodine is only acquired through the diet and absorbed in the digestive tract as the inorganic anion iodide [11].

Its adequate supplementation decreases endemic goitre and may achieve the eradication of IDD [1, 12].

Surface waters are regarded as the best index of the local environment's iodine status, particularly since iodine in water represents the bioavailable form of the element [13].

Some countries still lack general population data on IN, even within the European Union, as in Portugal [5, 14, 15], where the only national study on IN is limited to the population group of pregnant women [16], showing ID occurring throughout the entire country, with a global nationwide median UIC of 82.5 µg/L.

This study aims at evaluating the IN of the general population from the inland region of BI, in Portugal, never previously investigated. We measured general determinants of iodine nutrition status to include parameters of: UIC, the thyroid histology pattern and the largest surface water reservoir IC, none of them previously documented or reported. We surmised this would provide needed data to identify treatment strategies.

Materials and methods

Study design

This study was approved by the ethics committees of the Faculty of Health Sciences of the University of Beira Interior and of Centro Hospitalar Cova da Beira (Hospitals of Covilhã and Fundão).

The IN status of a cohort of the population of 211,205 [17] of the inland region of BI (beyond the mountain range of Serra da Estrela, which stretches across 115 km and has its highest peak at 1993 m above sea level [18]) is evaluated through the combination of three separate evidence parameters:

Urinary iodine excretion: population sample and measurements

Urinary iodine excretion was measured from a general population sample of 214 volunteers (131 females and 83 males), with ages ranging from 8 to 97 years (mean 51.5 years ± SD 20.74 years), residing in the study area of BI, not taking thyroid medication or supplements and that had not had thyroid surgery. These samples were collected over a 2-year period, in late 2012, 2013 and early 2014 from people residing in the region of Beira Interior on a random unselected sampling basis, including people from different walks of life, that consumed usual diets for the Portuguese population and following informed consent principles.

The assessment and monitoring of the IN status in population studies is best done through the determination of the median urinary iodine concentration (UIC) in random spot urine samples, in µg/L [11].

Random spot urine samples were obtained, UIC being measured by fast colorimetric method based on the original description method by Sandell and Kolthoff [19], and the median value was determined.

Histologic diagnosis

A retrospective evaluation of all thyroid histology reports over a 6-year period, between January 2002 and December 2007, from patients with a residential address from the study area of BI was performed. The histology reports were from patients residing in the region of BI. The standard method for general histology, with haematoxylin and eosin staining (H&E) and criteria according to the WHO revised classification of thyroid tumours, second edition [20], was used.

The time for this retrospective evaluation was arbitrarily chosen, the only objective being that of obtaining a representative sample of the thyroid nodular pathology from this study region.

The region of BI that lies beyond (to the east of) the Mountain range of Serra da Estrela was selected as the study area. The population of this area is 211,205 inhabitants [17].

Variability in histopathologic criteria has, however, been reported from different centres, particularly between local pathology centres and expert panels [21]. To try and overcome possible limitations, in the current study detailed description of the histology reports was fully evaluated and correlated with the pre-operative cytological report. In 3 cases, reassessment of the original slides was requested to obtain additional information concerning the diagnosis. These had pre-operative cytology reports of "suspicious for follicular neoplasm". Further slides from new cuts from these specimens were carefully checked for angio or capsular invasion, and as these were confirmed not to be present, the benign diagnosis was confirmed. This was performed at the same Histopathology Laboratory used in the study: Cedap—Coimbra (linked to the University Hospitals of Coimbra).

Iodine measurement in local water reservoir

Surface waters have been considered one of the best indices of an environment's iodine status [13]. The IC of the large main surface water reservoir of BI (Penhas-da-Saúde) situated at an altitude of 1557 m was analysed in samples collected in May and June 2013. These months were chosen so as to avoid possible dilution during the rainy season, predominantly mid-October till end of March, or concentration during the hot dry months of July through to end September. Samples were obtained from 1 m depth, in the middle of the reservoir so as to avoid any possible contamination and from the water side.

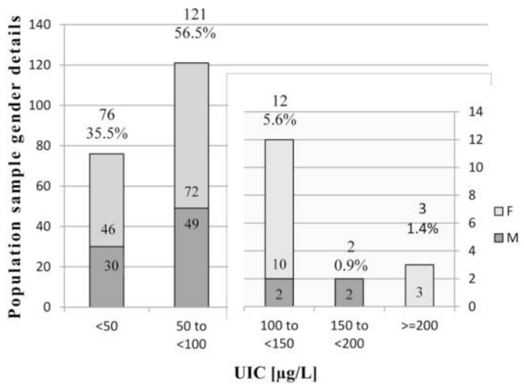

Fig. 1 Urinary iodine concentration UIC in population sample of 214 volunteers (131♀; 83♂) with ages ranging 8–97 years (median 52.0 years)

The IC was measured by the colorimetric method based on the description by Sandell and Kolthoff [19]. Absorbance was measured at a wavelength of 420 nm.

Statistical analysis

The thyroid pathology pattern is described in terms of absolute frequencies and percentages.

To evaluate the UIC, the mean value of three measurements taken on each collected sample was determined.

The median from these mean values was obtained as well as the 95 % confidence interval (CI) of the median (significance of the Kolmogorov–Smirnov test of normality <0.000). The iodine content of surface water is given as the mean ± standard deviation of the mean (significance of the Shapiro–Wilk test of normality = 0.945).

Most of the calculations were done in Excel [2007], using standard statistics formulae [22]. The normality tests were run in SPSS (version 21).

Results

Results of the UIC measured from a general population sample of 214 volunteers by spot urine sampling showed a median UIC of 62.6 µg/L (95 % CI 57.5–67.4 µg/L). Over a third of the samples ($n = 76/214$) had UIC values below 50 µg/L, and over 92 % had UIC below 100 µg/L ($n = 197/214$) (Fig. 1). This sample from 131 females and 83 males with a median age 52.0 years is representative of the general population of the study area of BI totalling

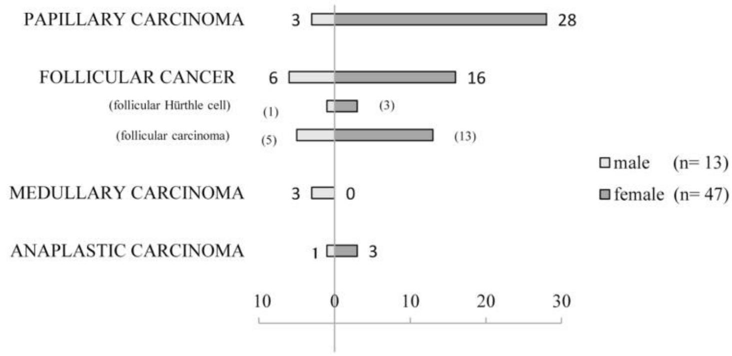

Fig. 2 Thyroid malignancies in Beira Interior, Portugal—gender distribution

Table 1 Thyroid neoplasias (histology) in Beira Interior, Portugal—age distribution

	Age (in years)				
	Mean	Minimum	Maximum	SD	*n*
Papillary carcinoma	57.0	27	83	14.3	31
Follicular adenoma	51.0	15	83	16.6	50
Follicular Hürthle cell	55.0	26	81	24.1	4
Follicular carcinoma	53.4	28	76	16.6	18
Medullary carcinoma	51.7	40	62	11.1	3
Anaplastic carcinoma	72.5	54	82	13.2	4

211,205 with a median age of 54.1 years and a comparable female predominance [17].

A total of 279 patients (242 females and 37 males) originating from the study area of BI region during the 6-year study period (January 2002 to December 2007) had thyroid surgery for thyroid nodular pathology. Their histology reports were assessed and used for the purpose of evaluating the relative incidence of the different types of thyroid nodular pathology in the study area. Published data relating to the total nationwide number of thyroidectomies in the USA have shown a steady increase from 66,864 in 1996 to 130,216 in 2011 [23].

In the benign pathology group, there were 219 patients: 143 multinodular goitres, 15 benign colloid nodules, 50 follicular adenomas, 10 thyroiditis and 1 Graves' disease.

Analysing the thyroid neoplasms, there were in total 110, made up of 50 follicular adenomas (as mentioned above) and 60 histologic diagnoses of malignancies (21.5% of the total 279 patients).

These thyroid malignancies were: 31 papillary TC, 22 follicular TC (18 follicular carcinomas and 4 Hürthle cell tumours), 3 medullary thyroid cancers and 4 anaplastic TC (Fig. 2).

The carcinomas of follicular cell origin (papillary, follicular and anaplastic) displayed a large female gender predominance of 82.5% and an overall female to male ratio of 4.7 (47/10).

In the medullary carcinomas (perifollicular or C cell origin), the opposite sex predominance was seen (0/3) although this disparity might be attributable to the relatively small numbers documented.

Except for the anaplastic TC, which had a mean age of 72.5 years, the ages for all the other cancer types reflect a fairly similar mean range in the fifth decade of life, ranging from the age of 26 to the age of 83 years (Table 1).

The papillary-to-follicular (P/F) carcinoma ratio was $31/22 = 1.41$ (95 % CI 0.82–2.43).

Measurements of the IC of the main surface water reservoir for BI (Penhas-da-Saúde) were taken in a total of 13 samples from the middle of the reservoir. The results of all samples were comparable, and their mean value was 14.33 µg/L ± 0.36 µg/L.

Discussion

The recommended daily iodine intake [25] is: ≥120 µg for schoolchildren (6–12 years); ≥150 µg for adults; and 250 µg for pregnant or lactating women. Iodine deficiency occurs when the median UIC is below 100 µg/L [24, 25]. From Portugal, there is still no general population data on IN [5, 14, 15].

The current study represents the first general population evaluation of IN in Portugal, from the inland region of BI. This was performed using the accepted population study method of the median UIC, through random spot urine samples [11]. Results showed a median UIC for the general population sample from BI of 62.6 µg/L, with the added statistical validation of the 95% CI ranges that lie well below the reference value of 100 µg/L. Over 92 % of the population sample had a low UIC of less than 100 µg/L.

The only nationwide study on IN published from Portugal was done on the selected population group of pregnant women [16]. This study showed ID occurring throughout the entire country, with a global nationwide median UIC of 82.5 µg/L, the result for BI being a median UIC 67.6 µg/L which is similar to the result of the current general population study: median UIC 62.6 µg/L. This could, arguably, indicate that the results in this only national IN study from Portugal are likely to also reflect the general population IN levels.

In support of this likely hypothesis, that the iodine intake is similar for the entire family is the fact that, in Portugal, the majority of the population tends to have meals that are prepared for the entire family by a matriarch of the family. This likely hypothesis should, nevertheless, still be further proven in a National general population study on IN, which hopefully will be spearheaded by the publication of the results from this present study.

Surface waters have been regarded as the best index of an environment's iodine status, iodine in water representing the bioavailable form of the element [13]. The iodine content of the main surface water reservoir for BI had a mean value of 14.33 µg/L. There is no data available on iodine content of water reservoirs to enable establishing reference values.

The current study documents for the first time the IC of the large main water reservoir of Penhas-da-Saúde, one of the largest surface water reservoirs in Portugal.

The adverse effects of iodine deficiency have been shown to include variable degrees of intellectual impairment [26], demonstrable neuropsychointellectual deficits [6, 27], impaired reproductive potential [28], development of goitre and thyroid nodular pathology [12] and an increase in the prevalence of thyroid cancer [10, 29]. The occurrence of higher percentages of the more aggressive follicular and anaplastic subtypes of thyroid cancer in iodine-deficient areas has also been shown [10, 20] and recently confirmed in a comprehensive review on iodine intake and risk factors for thyroid cancer [30].

The results of the current study from BI, with a median UIC of 62.6.0 µg/L, in which over a third of the population samples had a value of less than 50 µg/L and over 4 % less than 20 µg/L, confirm the persisting problem of iodine deficiency. These results emphasise a need for adopting more active policies, if the elimination of ID is to be achieved.

In iodine-deficient areas, papillary-to-follicular carcinoma ratios tend to vary from 0.19:1 to 1.7:1 [31], in contrast with high iodine consumption areas that have ratios varying from 3.4:1 to 6.5:1 [31]. In our current study, the histology pattern of thyroid nodular pathology displayed in the region of BI is typical of iodine-deficient areas with the papillary-to-follicular carcinoma ratio being relatively close to 1 [1.41 (=31/22)] and a fairly high percentage of anaplastic carcinomas [10] of 6.7 % (4 from 60 thyroid malignancies).

In the only nationwide study on IN from Portugal, which evaluated the population group of pregnant women [16], significant iodine deficiency was shown to exist nationwide, with a median UIC of 82.5 µg/L. The lowest value was documented from the mid-Atlantic islands of Açores, with a UIC of 50.0 µg/L, followed by the region of BI with a median UIC of 67.6 µg/L, the lowest value in Continental Portugal.

The low UIC result shown from the mid-Atlantic islands of Açores, where sea-based nutrition would be easily available, highlights the need for adequate population information as to the negative consequences of ID.

Influenced by the Portuguese Endocrine Society, the Directorate General of Health (Portugal) published guidelines in August 2013 [32] recommending (but not prescribing) that pregnant, breastfeeding women and in the pre-pregnancy age should be given a daily supplement of potassium iodate in a dose of 150–200 µg. This value is, however, still inferior to the recommended amount by the WHO of 250 µg daily [24].

The successful correction of ID requires easy and affordable access to iodised salt by the general population.

This is documented as having been done in China through the combination of population educational policies and a legislative ruling, in 1978, that the cost of iodization of salt be borne by the government, as this would be to the benefit of the entire country's population [33]. The ongoing sub-national population monitoring for iodine nutrition combined with the implementation of the salt iodization programs also contributed to China's well-documented and continued successful strategies [34].

Another example of the successful elimination of ID comes from South Africa [35] where legislation enforcing the iodisation of all manufactured food grade salt was introduced in 1995 [36].

A countywide survey on the availability of different types of salt in Continental European Portugal, undertaken by the first author of the current study, revealed that whilst non-iodised salt exists in several brands at supermarkets, iodised salt (specifying the iodine content) is more rarely available. When available, its price is more than double that of non-iodised salt (unpublished results). Enlisting a proactive behaviour from a well-informed general population as to the serious adverse effects of ID could also be important in achieving its successful elimination.

Worldwide other significant improvements have occurred in the elimination of ID through the introduction of programs for access to iodised salt [2], whereas in the Americas the percentage of households having access to iodised salt is reported to exceed 90 % [38]; in Europe, this is a mere 27 % [37]. Since Portugal has salt-producing facilities [38], legislation requiring all commercially available salt to have a set amount of iodine could be implemented. Current legislation in Portugal relating to salt iodization was adopted in 1969, on a voluntary basis [38]. Furthermore, with the majority of the Portuguese population living close to the sea and having relatively easy access to sea generated food products, naturally iodine containing, the elimination of ID could also be facilitated by people being adequately informed about ID.

Conclusions

This study evaluates for the first time the general population's IN in Portugal, in the region of BI, using combined evidence of: the UIC of a general population sample and a retrospective 6-year evaluation of the thyroid histology pattern. The findings demonstrate the existence of significant iodine deficiency: median UIC 62.6 μg/L; 92 % samples having UIC < 100 μg/L; and 6-year thyroid histology pattern, characteristic of ID: papillary-to-follicular carcinoma ratio close to 1 (1.4) and a relatively high number of anaplastic carcinomas (6.7 %). This ID problem could be corrected by having affordable iodised salt generally available, promoting sea-based nutrition and eliciting a proactive population behaviour in a well-informed public as to the negative consequences of ID.

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Authors' contributions JECS contributed to the design of the study, the major writing of the manuscript as well as the acquisition of most of the data; he coordinated the statistical design and the data interpretation. JLR contributed to the writing of the manuscript, the sequence alignment, as well as to its critical revision and correction. MF contributed to the elaboration and writing of the statistical analysis, as well as to the elaboration and design of table and figures, and participated in the sequence alignment critical revision and correction of the manuscript. CPF designed, performed and supervised the laboratory evaluation of urinary iodine concentration (UIC) and the iodine measurement in local water reservoir, as well as writing the sections relating to this part of the manuscript. PC performed most of the laboratory evaluation of the UIC. IMC contributed to the sequence alignment, critical revision and correction of the manuscript. MCB participated in the overall supervision and critical evaluation of the study, as well as the critical revision and correction of the study. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that no competing financial interests exist.

Ethical approval This study was approved by the ethics committees of the Faculty of Health Sciences of the University of Beira Interior and of Centro Hospitalar Cova da Beira (Hospitals of Covilhã and Fundão), research involving human participants being performed in accordance with ethical standards as laid down in the 1964 Declaration of Helsinki, its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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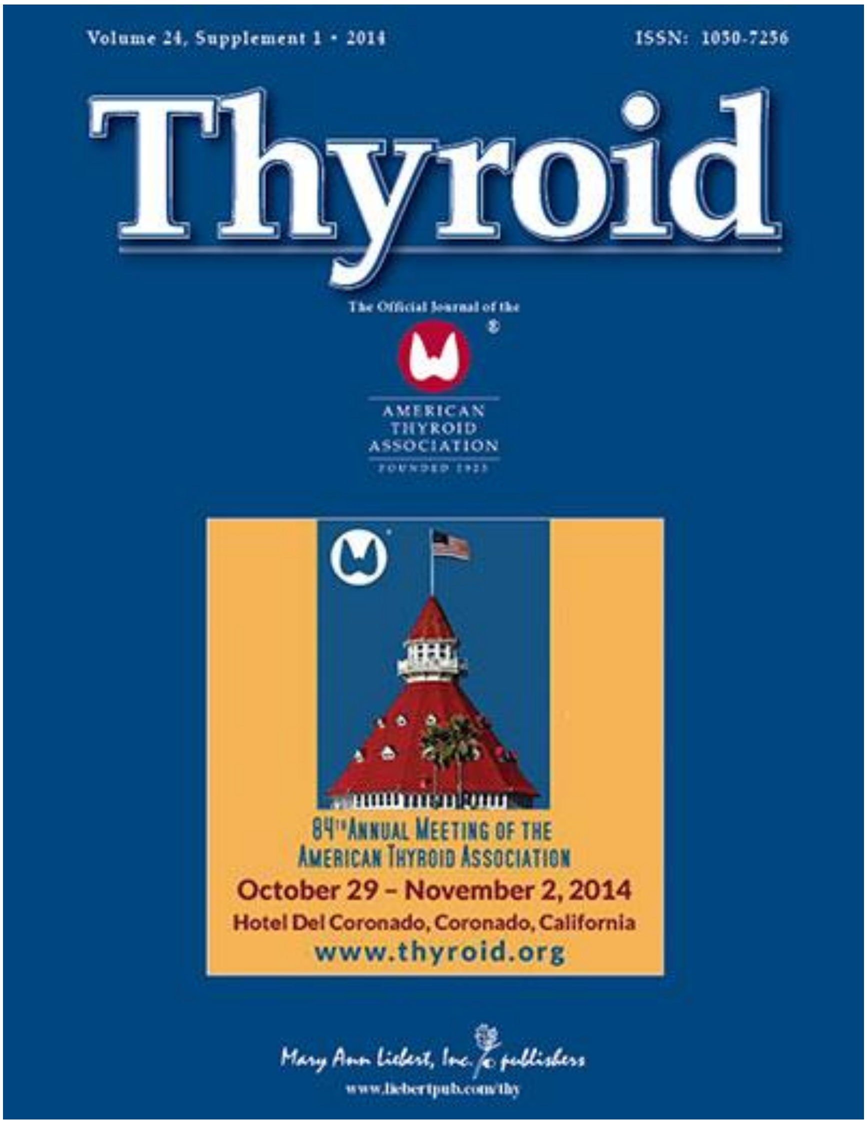
1) Santos JEC, Freitas M, Fonseca CP, Castilho P, Carreira IM, Castelo Branco M. Thyroid 2014; 24(S1):A-70.



October 2014
Vol. 24, No. S1

Iodine *Deficiency* and Thyroid Nodular Pathology- Epidemiological and Cancer Characteristics in Different Populations.

Santos JEC, Freitas M, Fonseca CP, Castilho P, Carreira IM, Castelo Branco M.www.thyroid.org. Thyroid 2014; 24(S1):A-70.October 2014; Vol. 24, No. S1

Poster 175

Thyroid Cancer Friday Poster Clinical

SEVERE IODINE DEFICIENCY IN AN INLAND REGION OF PORTUGAL-EVALUATION OF THE THYROID NODULAR PATHOLOGY AND ASSESSMENT OF THE IODINE NUTRITION

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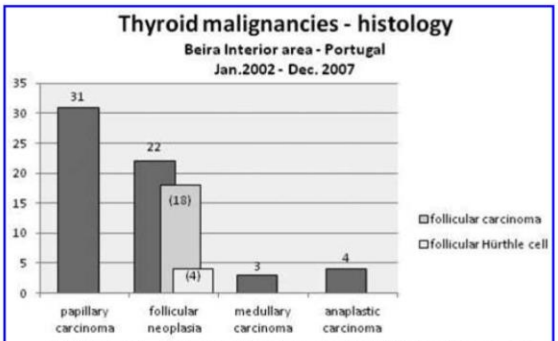
The universal endorsement in 1990 of the goal of virtual elimination of iodine deficiency (ID) by the year 2000 has still not been achieved in several signatory countries. Two billion people worldwide (over two million school age children), remain ID. In Europe there are still countries with no national population data on iodine nutrition, amongst which, Portugal. The current study aims at evaluating the iodine nutrition in the inland mountainous region of Beira Interior (BI), in Portugal.

Determination of urinary iodine concentration (UIC) from a general population sample of 192 volunteers from BI (2012/2013/2014), measurements of the iodine content of the largest water reservoir of the region of BI and evaluation of the thyroid histology pattern (6 year period). Urinary iodine excretion measurements: 91.7% of the general population was iodine deficient with a median UIC 63.50 μg/L. The mean iodine content of the main surface water reservoir for BI was 0.584 μg/mL. From 279 histology reports available the incidence of the different types of thyroid nodular pathology in BI was established. There were 60 histologic diagnoses of malignancy: 31 papillary carcinomas, 22 follicular cancers (18 follicular carcinoma and 4 Hürthle cell tumour), 3 medullary carcinomas, and 4 anaplastic carcinomas. The papillary to follicular carcinoma ratio was 31/22 = 1.41. The findings in what is, to our knowledge, the first general population study from the inland region of BI, in Portugal, demonstrate significant ID. Ample public information combined with educational programs as to the negative consequences of ID, availability of iodized salt at affordable prices and the promotion of seafood consumption would assist in solving the problem.



Histological diagnosis - Thyroid malignancies - Beira Interior: 6 year period - (January 2002 – December 2007).
The types of follicular cancer are discriminated.

2) Thyroid Cancer Friday Poster Clinical

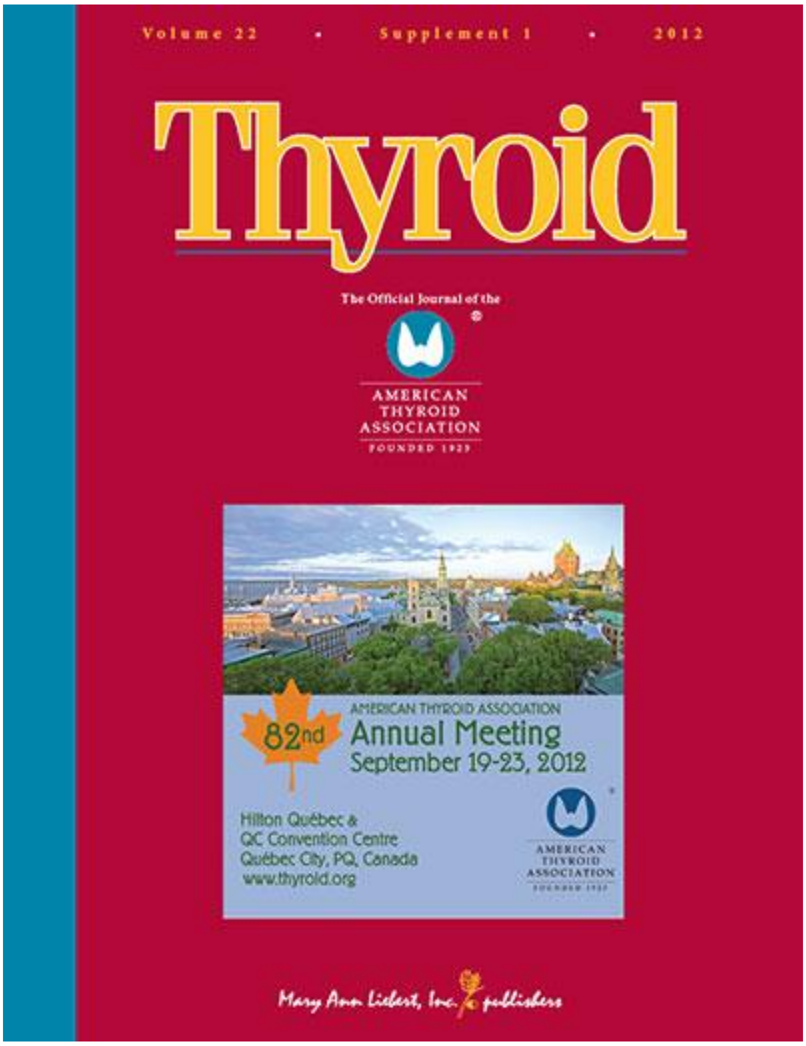
Santos J, Freitas M, Fonseca CP, Castelo Branco M. Poster 165

Thyroid Cancer Friday Poster Clinical

IODINE DEFICIENCY IN AN INLAND AREA OF PORTUGAL:

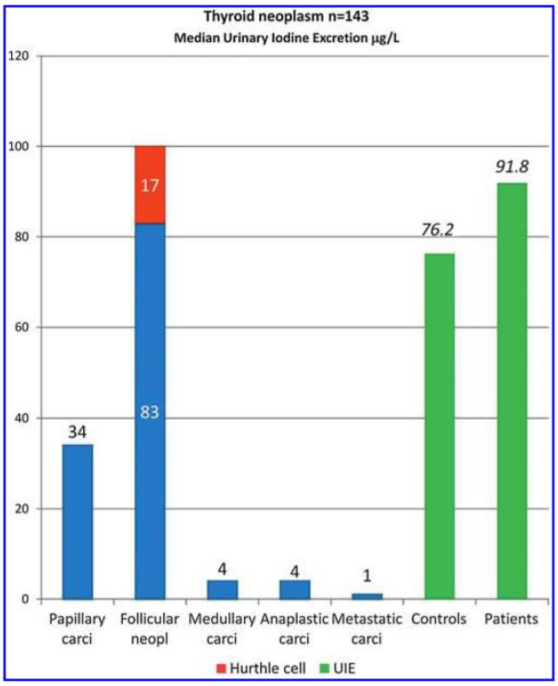
THE PATTERN OF THYROID NODULAR PATHOLOGY

Santos J, Freitas M, Fonseca CP, Castelo Branco M. Iodine Deficiency in na Inland Area of Portugal. Thyroid 2012; 22(S1); A67.



September 2012

Vol. 22, No. S1



POSTER 165. Sample of 861 patients (Jan. 2002–Jan. 2008).

Poster 165

Thyroid Cancer Friday Poster Clinical

IODINE DEFICIENCY IN AN INLAND AREA OF PORTUGAL: THE PATTERN OF THYROID NODULAR PATHOLOGY

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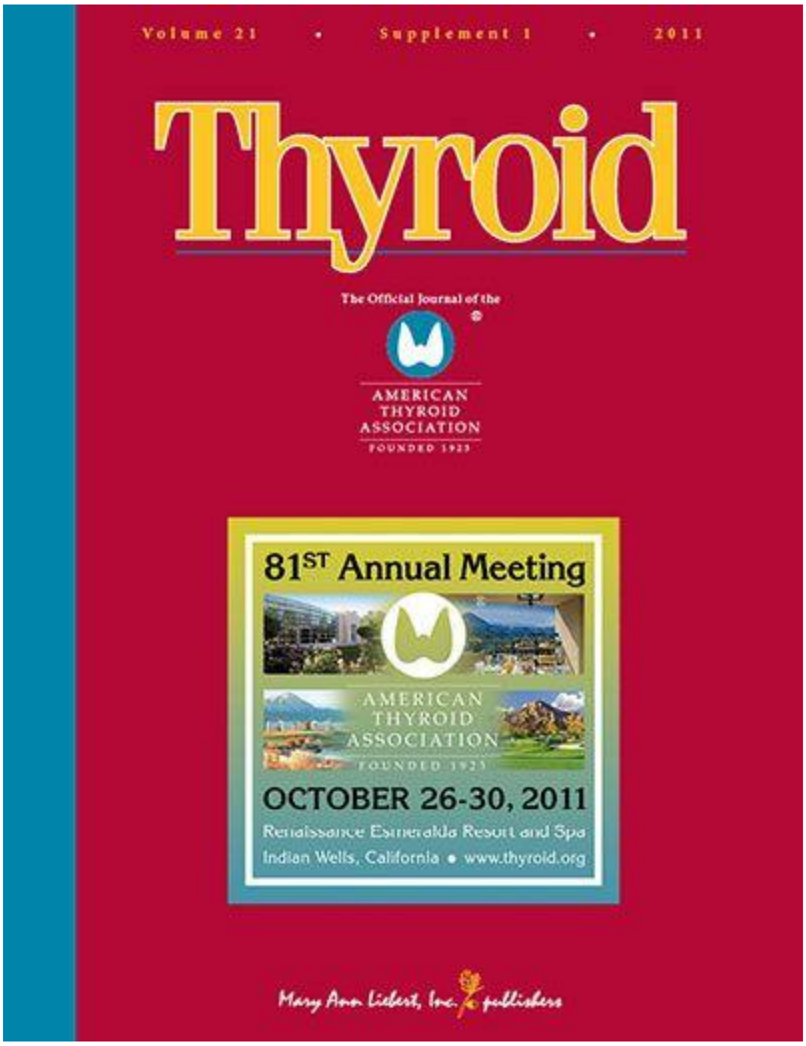
The iodine ingestion of the population determines the pattern and prevalence of thyroid pathology. Iodine deficiency (ID) is influenced by many factors: the availability and cost of certain nutrients, cultural and lifestyle patterns, availability of iodized supplements or salt, and public education as to all different adverse consequences of ID. Close to 2 billion people worldwide are ID. ID may lead to a sustained increase in TSH, causing microscopic and macroscopic heterogeneity in the thyroid, increased incidence of thyroid cancer, and a rise in the ratio of follicular to papillary neoplasia. Urinary iodine excretion (UIE) is the most accurate method for assessing the iodine nutritional status of a population. Limited data, only recently published, show that the whole of Portugal to be ID.

Evaluation of pattern of thyroid nodular pathology in the most ID area of Continental Portugal, Beira Interior (BI), lying beyond high mountain ranges; population 211,189 (Census 2011). Data from all major referral Hospitals from BI and from the 3 existing Portuguese Oncology Institutes based on Cytological and Histological reports done from start of 2002 to start of 2008, from patients residing in BI assessed. UIE was measured by spectrophotometry (Sandell–Kolthoff reaction) in 13 patients presenting with thyroid nodules at the main Teaching Hospital of BI over a 3-month period and in 10 controls balanced for age and sex.

UIE of all 23 samples from MTHBI confirmed the recently published data finding of BI as significantly ID, both in the control group and in patients: C Median UIE = 76.15 μg/L; P Median UIE = 91.83 μg/L. Total of 869 patients evaluated over 6 years: 607 with cytology reports and 278 histology. From the 869 patients, 143 had thyroid neoplasm: 34 had papillary carcinoma, 100 follicular neoplasm, 4 medullary carcinoma, 4 anaplastic/undifferentiated carcinoma, and 1 metastatic carcinoma to the thyroid.

The inland area of Portugal studied of BI is significantly ID as reflected by the inversion of the usual ratio of the papillary/follicular ratio. Correction of this problem can only come from ample public education that leads to pressure on politicians to adopt Universal Salt Iodization.

3) Santos JEC, Freitas M, Marques A. Thyroid Nodular Pathology in na Iodine Deficient Area in the Interior of Portugal. Thyroid 2011;21(S1);A73-A74. .



October 2011
Vol. 21, No. S1

Poster 172

Thyroid Nodules and Goiter Friday Poster Clinical

THYROID NODULAR PATHOLOGY IN AN IODINE DEFICIENT AREA OF THE INTERIOR OF PORTUGAL

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The prevalence and pathology patern of iodine deficiency disorders (IDD) are related to dietary iodine intake. Two bilion people worldwide are iodine deficient (ID). Iodine is essential for thyroid hormones production. They regulate metabolic processes in most cells and are important in the development of most organs, particularly the brain. ID leads to sustained increased production of TSH and development of microscopic and macroscopic heterogeneity. ID:increased incidence of thyroid cancer and rise in the ratio of follicular to papillary neoplasia occurs. Mandatory iodization of table salt leads to a decrease in goitres and eradicates IDD. Public education as to adverse effects of ID is needed. Limited data available, shows the whole of Portugal is ID, particularly areas of the interior. Fine needle biopsy (FNB), aspiration or non-aspiration methods is the investigation of choice for thyroid nodules.

Aim of study: relative incidence of different types of thyroid nodular pathology in the most ID area: Beira Interior (BI); population, 232,000 inhabitants (2001 Census). All thyroid cytology and histology reports relating to 6 years from the major referral Hospitals for the study area of BI (MRHBI) were obtained. Aiming at being epidemiologically thorough gathering all clinically manifest patients with thyroid nodular disease in BI, MRHBI data was complemented with equivalent data, Nationally from the 3 existing Portuguese Oncology Institutes. Total of 869 patients evaluated: 607 of them having had FNB (cytology reports) and 278 had histology reports.

From the 869 patients that had thyroid nodular disease 143 had thyroid neoplasms (TNeo). From the 143 patients with TNeo 34 patients had papillary carcinoma and 100 follicular neoplasms. In the 143 TNeo were 4 medullary carcinomas, 4 anaplastic carcinomas and 1 metastatic carcinoma.

- Portugal is iodine deficient.
- 6 year study period: incidence of thyroid nodular disease in the study area of Beira Interior, was 3.75 per 1,000 population.
- The incidence of thyroid neoplasms was 0.62 per 1,000.
- The follicular neoplasms to papillary carcinoma ratio, in the study period, was 100/34.
- Mandatory iodine suplementation should be implemented in Portugal.

Poster Presentations:

1) Poster Presentation at the 84th Annual Meeting of the American Thyroid Association

UNIVERSIDADE DA BEIRA INTERIOR Covilhã | Portugal — Faculdade de Ciências da Saúde — Centro de Investigação em Ciências da Saúde (Health Sciences Research Centre) — Centro Hospitalar Cova da Beira, E.P.E.

IODINE DEFICIENCY A PERSISTENT WORLDWIDE PROBLEM - ASSESSMENT OF THE IODINE NUTRITION AND EVALUATION OF THYROID NODULAR PATHOLOGY IN PORTUGAL.

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Introduction

The goal of eliminating iodine deficiency (ID) by the year 2000, endorsed by all attending countries to the World Summit for Children, September 30th 1990, attended by 71 Heads of State and representatives of 159 Governments at the United Nations, New York, has still not been achieved in several countries. More than 2 billion people worldwide (over 260 million million school age children remain ID. In Europe there are still countries, such as Portugal, without national general population data on iodine nutrition.

Iodine is essential for thyroid hormone production, which regulate metabolic processes in most cells and are important in the development of most organs, particularly the brain.

Objective

The current study aims at evaluating the iodine nutrition of the general population of the inland mountainous region of Beira Interior (BI), in Portugal, using three levels of evidence.

Methods

1. Determination of urinary iodine concentration (UIC) from a population sample of 192 volunteers from BI over a two year period.
2. Measurements of iodine content of the largest surface water reservoir of BI .
3. Evaluation of the thyroid histology pattern from all patients with residential address in the study area of BI, over a 6 year period.

Results

Urinary iodine concentration measurements: median 63.50µg/L. Over 91% was ID (95%CI: 87.8% to 95.6%).

The mean iodine content of the main surface water reservoir for BI was 0.584µg/mL.

Thyroid histology pattern evaluated from a total of 279 patients (242♀ ; 37♂)

60 histological diagnoses of malignancy: 31 papillary carcinomas, 22 follicular cancers (18 follicular carcinoma and 4 Hürthle cell tumour), 3 medullary carcinomas and 4 anaplastic carcinomas.

Papillary to follicular carcinoma ratio = 1.41 (31/22)

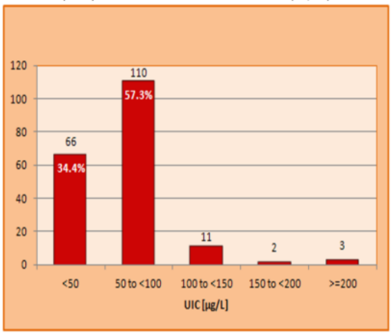

Fig. 1 - Urinary Iodine Concentration (UIC) in population sample of 192 volunteers (118♀ ; 74♂) with ages ranging 8 to 97 years (median: 54.0 years).

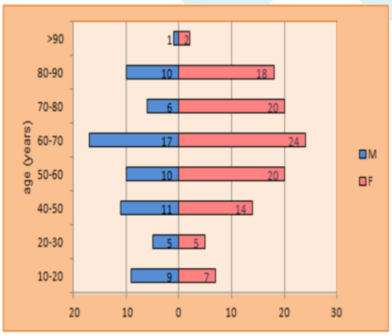

Fig.2 Age and gender spectra in UIC population sample of 192 volunteers

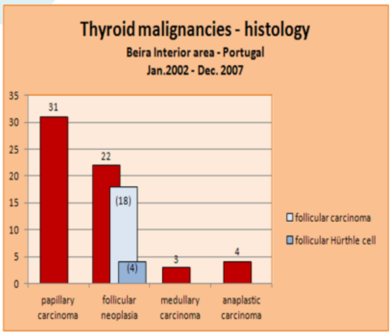

Fig.3 - Histological diagnosis - Thyroid malignancies - Beira Interior: 6 year period (January 2002 – December 2007).
The types of follicular cancer are discriminated.

Conclusions:

The findings of this first general population study from the inland region of Beira Interior demonstrate significant iodine deficiency with over 91% of the population being ID.

The mean iodine content of the main surface water reservoir (regarded as the best index of an environment iodine status) for BI was extremely low : 0.584µg/mL.

The retrospective 6 year evaluation of the thyroid histology pattern for BI is indicative of ID with a relatively high rate of follicular and anaplastic carcinomas and a papillary to follicular carcinoma ratio that was close to 1.

Ample public information and educational programs highlighting the negative consequences of ID, combined with availability of iodized salt at affordable prices and the promotion of seafood consumption would assist in eliminating ID.

Presentation 31/10/2014 – 84th Annual Meeting of the American Thyroid Association - San Diego, California, U.S.A.

Coronado, San Diego, U.S.A. October 31st 2014

2) Poster Presentation at the 82nd Annual Meeting of the American Thyroid Association

Québec City, Canada. September 21st 2012.

UNIVERSIDADE DA BEIRA INTERIOR Covilhã | Portugal — FCS Faculdade de Ciências da Saúde — Centro de Investigação em Ciências da Saúde Health Sciences Research Centre — Centro Hospitalar Cova da Beira, E.P.E.

IODINE DEFICIENCY IN AN INLAND AREA OF PORTUGAL: THE PATTERN OF THYROID NODULAR PATHOLOGY.

José Eduardo Santos[1, 2, 4]; Miguel Freitas[3]; Carla Fonseca [1,2]; Miguel Castelo Branco [1, 2, 4]

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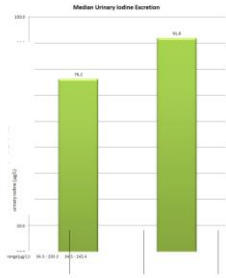
4. Centro Hospitalar Cova da Beira, Covilhã, Portugal.

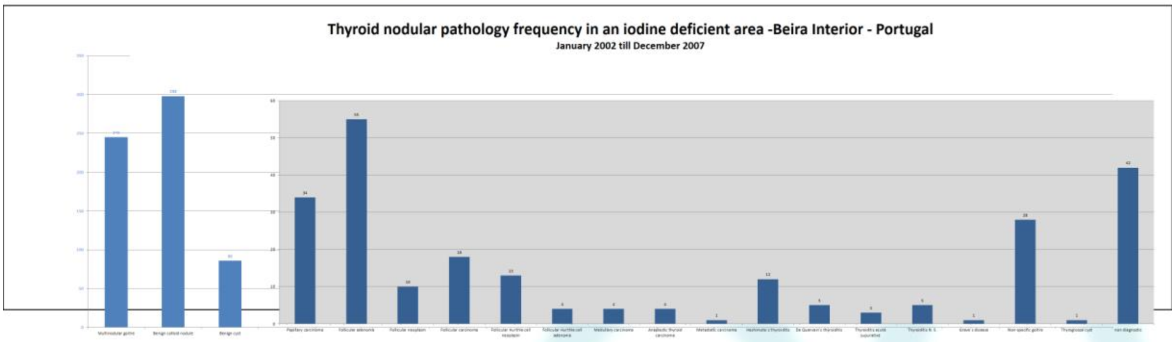
Introduction

The prevalence and pathology pattern of iodine deficiency related disorders (IDRD) reflects the dietary intake of the population being evaluated. Iodine deficiency (ID) is influenced by many factors: Availability and cost of certain nutrients; Level of education of the population; Culture and lifestyle; Availability and cost of iodised salt and supplements. An important factor is the level of public information and education as to all the possible adverse consequences of ID: Increased miscarriage rate; Mongolism or varying degrees of mental impairment and physical stunting of the offspring; Intellectual impairment with demonstrable degrees of neuropsychointelectual deficit; damaged reproduction potential; Goitre and thyroid nodular pathology; Possible increase in the prevalence of thyroid cancer.
The 1990 World Health Assembly and the World Summit for Children had Universal endorsement of a Declaration that aimed at the virtual elimination of iodine deficiency. In spite of this there are still close to 2 billion people worldwide that are ID.
Iodine deficiency may lead to a sustained increase in TSH, causing microscopic and macroscopic heterogeneity in the thyroid, possible increased incidence of thyroid cancer and a rise in the ratio of follicular to papillary thyroid neoplasia.
There has been no National data available from Portugal on the levels of iodine nutrition. Limited National data recently published shows the whole of Portugal to be ID.
Urinary Iodine Excretion (UIE) is the most accurate method for assessing the iodine nutritional status of the population.



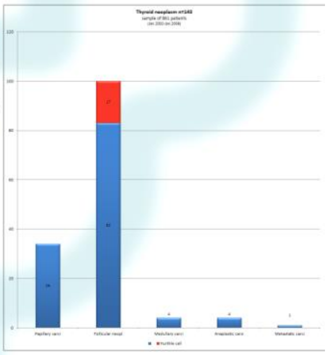
Methods

Evaluation of the pattern of thyroid nodular pathology in the most ID area of Continental Portugal, Beira Interior (BI) - population 211,189 (Census 2011), was made.
Data relating to all Cytological and Histopathologic thyroid reports from the major referral Hospitals from BI and from the 3 existing Portuguese Oncology Institutes relating to patients residing in BI was assessed.
UIE was measured by spectrophotometry (Sandell-Kolthoff reaction) in 13 patients presenting with thyroid nodules at the main Teaching Hospital of BI (MTHBI) over a 3 month period and in 10 controls balanced for age and sex.



Results

A total of 869 patients were evaluated over the 6 year period - January 2002 to December 2007: 607 patients had cytology reports and 278 Histology. From the 869 patients 143 had thyroid neoplasm's: 34 had papillary carcinoma, 100 follicular neoplasm, 4 medullary carcinoma, 4 anaplastic/undifferentiated carcinoma and 1 metastatic carcinoma to the thyroid
UIE of all 23 samples from the MTHBI confirmed the recently published data that showed that BI has a population that is significantly ID. This was shown in both the patient group (median UIE=91.8µg/L) and the control group (median UIE= 76.2µg/L)



Conclusions

-The inland area of Portugal studied of Beira Interior is significantly Iodine Deficient as reflected by the inversion of the usual ratio of papillary to follicular neoplasia

- Correction of this problem will only come from ample public education that leads to pressure on politicians to adopt Universal Salt Iodisation.

3) Poster Presentation at the 81st Annual Meeting of the American Thyroid Association

Indian Wells, California, U.S.A. October 28th 2011.

UNIVERSIDADE DA BEIRA INTERIOR Covilhã | Portugal — FCS Faculdade de Ciências da Saúde — Centro de Investigação em Ciências da Saúde Health Sciences Research Centre — Centro Hospitalar Cova da Beira, E.P.E.

IODINE DEFICIENCY a PERSISTENT PROBLEM WORLDWIDE- ASSESSMENT of the IODINE NUTRITION and EVALUATION of THYROID NODULAR PATHOLOGY IN PORTUGAL.

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Introduction

The goal of eliminating iodine deficiency (ID) by the year 2000, endorsed by all attending countries to the World Summit for Children, September 30th 1990, attended by 71 Heads of State and representatives of 159 Governments at the United Nations , New York, has still not been achieved in several countries. More than 2 billion people worldwide (over 260 million million school age children remain ID. In Europe there are still countries, such as Portugal, without national general population data on iodine nutrition.
Iodine is essential for thyroid hormone production, which regulate metabolic processes in most cells and are important in the development of most organs, particularly the brain.

ID leads to sustained increased production of TSH which is associated with the development of microscopic and macroscopic heterogeneity and has been shown to be associated with increased incidence of thyroid cancer as well as with a rise in the ratio of follicular to papillary neoplasia.

Mandatory iodization of table salt coupled with public education as to the adverse effects of ID leads to a decrease in the incidence of goitres and to the eradication of IDD.

Methods:

Aim of study- the relative incidence of diferent types of thyroid nodular pathology in the most ID deficient area of Continental Portugal: Beira Interior (BI) – Population 232,000 (2001 Census), equal male to female ratio up to the age of 65 years.

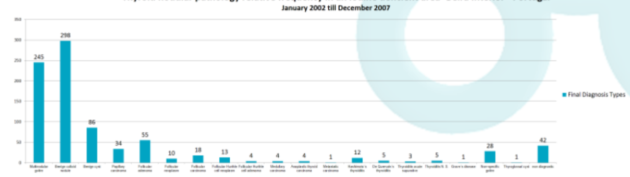
All thyroid cytology and histology reports relating to the 6 year period (January 2002 to January 2008) from the Major Referral Hospitals for the study area of BI (MRHBI) were obtained. Aiming at being epidemiologically thorough all clinically manifest patients with thyroid nodular disease in the area of BI, MRHBI data was complemented with all the available equivalent data, on a National level, from the 3 existing Portuguese Oncology Institutes.

A total of 869 patients were evaluated, 607 of them having had Fine Needle Biopsy / cytology reports and 278 histology reports. Some patients had both investigations.

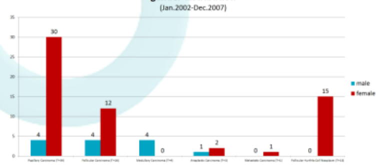
Results:

From the 869 patients that had thyroid nodular pathology 143 had thyroid neoplasms. From these 143: 34 had papillary carcinoma and 100 follicular neoplasms (15 Hurtle cell tumour and 16 follicular carcinoma); there were 4 medullary carcinomas, 3 anaplastic and 1 metastatic.

Thyroid nodular pathology relative frequency in an iodine deficient area -Beira Interior - Portugal
January 2002 till December 2007



Carcinoma and Hurthle Cell Neoplasm: gender distribution
(Jan.2002-Dec.2007)



Conclusions:

The findings of this first general population study from the inland region of Beira Interior demonstrate significant iodine deficiency with over 92% of the population being ID.
The mean iodine content of the main surface water reservoir (regarded as the best index of an environment iodine status) for BI was extremely low : 0.584μg/L.
The retrospective 6 year evaluation of the thyroid histology pattern for BI is indicative of ID with a relatively high rate of follicular and anaplastic carcinomas and a papillary to follicular carcinoma ratio that was close to 1.
Ample public information combined with educational programs as to the negative consequences of ID, combined with availability of iodized salt at affordable prices and the promotion of seafood consumption would assist eliminating ID.

Chapter 8

Conclusions and Future Perspectives

This study evaluates for the first time the iodine nutrition (IN) of the general population of the region of Beira Interior (BI), using combined evidence of UIC measurements in a population sample of 214 volunteers and the thyroid histology pattern evaluated over a 6 year period. There is evidence of the existence of significant iodine deficiency: median UIC 62.6µg/L (92% of samples had UIC <100µg/L) and a thyroid histology pattern typical of ID: papillary to follicular carcinoma ratio close to 1 (1.4) and a relatively high number of anaplastic carcinomas (6.7%).

From Portugal there is still no general population data on IN [43,45,66]. This data is important to identify and monitor treatment and corrective strategies.

A comparative evaluation of the IN through the thyroid histology pattern of the diverse populations of the Johannesburg, South Africa, region for 5 years (preceding the introduction of mandatory salt iodization in 1995) and the BI region in Portugal over 6 years, revealed similarities in the patterns in both areas, with a relatively high anaplastic thyroid carcinoma incidences and incidences of papillary to follicular carcinoma ratios showing overlapping 95% CI´s, also confirmed by the results of the chi-square calculations.

Iodine is acquired through the diet [49] being absorbed in the form of iodide. After its ingestion in food stuffs or dietary supplements. Iodine is nearly entirely absorbed (>90%) in the stomach and duodenum [50].

The realization by politicians of the serious consequences and negative economic impact to the country of a population that is ID is very important. It was based on that argument that legislation was adopted in China [76,82] and in South Africa [47]. As a result, policies of salt iodization were implemented that have been shown successful in both China [76,82] and in South Africa [48]. These government policies have resulted in iodized salt being available countrywide, at the same price as non iodized salt. The implementation of regular population monitoring for UIC is also routine in China [82] as part of an ongoing strategy for the non re-occurrence of the problem of ID. The examples of these success measures should be followed by all countries still experiencing the problem of iodine deficiency.

Portugal has salt producing facilities [77]. Legislation requiring all commercially available salt to have a set amount of iodine would be feasible. This measure would be more likely to succeed through an informed public opinion as to the serious negative consequences of ID so as to elicit a pro-active population behaviour. The promotion of sea based nutrition, naturally iodine containing could also assist in the elimination of ID in a well informed public.

Properly planned public information campaigns, organized with the support of all the media (audio, audio-visual and print) would be important in achieving the goal of eliminating iodine deficiency.

There is a need for a national study on the general population iodine nutrition done through the random spot urine samples measured for UIC. This information would allow for effective planning of loco-regional and national treatment strategies.

Even after the eventual elimination of ID in Portugal, regular population monitoring through UIC estimations would be important to prevent the re-occurrence of the problem.

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Appendix

A very relevant publication in the field of Thyroid Nodular Pathology and its diagnostic approach is the article that represents the first ever literature description and publication of a new technique in Cytology. This technique was developed in an attempt to overcome the problem of haemorrhagic specimens frequently encountered with the classical Fine Needle Aspiration Biopsy (FNAB). Haemorrhagic specimens would dilute and obscure the follicular cell samples and could interfere with the diagnostic ability of the Cytopathogist to achieve a diagnosis.

As mentioned in the text of the article the term employed for the title of this first description of this new technique of “nonaspiration fine needle cytology” was chosen for its descriptive nature, although as mentioned in the text of the article the authors refered to it as “cappilary technique”. This term has, in fact become the current used name for this technique in the literature and in the frequently published articles that refer to this technique. It is reported to have been cited up to late September 2015 in 112 articles in the literature. It is also referred to and cited in leading Textbooks such as “Werner and Ingbar The Thyroid A Fundamental and Clinical Text” being mentioned in the Ninth Edition, Chapter 73 “Clinical Evaluation and Management of Solitary Thyroid Nodules” page 999 and following with the Reference being found in page 1009. In the Textbook “Surgery of the Thyroid and Parathyroid Glands” by Gregory W. Randolph, 2003, the technique is described in Chapter 12: “Fine Needle Aspiration Biopsy of the Thyroid, by Hossein Gharib and John R. Goellner page 150 and in the References section mentioned in page 159.

Santos JEC, Leiman G. Nonaspiration Fine Needle Cytology – Application of a New Technique to Nodular Thyroid Disease. Acta Cytologica 1988; 32:353-356.

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Nonaspiration Fine Needle Cytology

Application of a New Technique to Nodular Thyroid Disease

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Gladwyn Leiman, M.B.B.Ch., F.I.A.C.

The basic principle underlying fine needle aspiration (FNA) is the aspiration of cellular material from target masses, often utilizing fairly high suction pressures. The procedure requires a needle and a syringe, advisedly held in a syringe holder, enabling single-handed suction to be exercised. Mastery of the technique is variable, with few operators acquiring consistent skill. A new technique, pioneered in France but essentially unpublicized, eliminates active aspiration, replacing it by the principle of capillary suction of fluid or semifluid material into a thin channel (a fine needle). This nonaspiration sampling method was tested in a consecutive series of 50 solid thyroid nodules. Simultaneously performed conventional FNAs served as controls. Cell samples were cytologically assessed as unsuitable, diagnostic/adequate or diagnostic/superior, without knowledge of the sampling method employed. Diagnostically superior specimens were obtained significantly more frequently by the nonaspiration technique in 36 benign lesions and 13 neoplasms. The method of nonaspiration fine needle cytology ("cytopuncture") is described and illustrated, and the implications for its use in other sites are discussed.

Fine needle aspiration (FNA) is an established diagnostic modality, which has expanded in recent years to include sampling of masses in virtually any body site, whether superficial or deep, palpable or nonpalpable. The aspiration technique generally employed is that of the Karolinska Institute, as described by Löwhagen et al,[2] for example. This involves firm attachment of the aspirating needle to a syringe, usually held in a specially designed syringe holder, which enables single-handed strong suction to be applied. Particularly in vascular organs or hemorrhagic tumors, this technique is not infrequently complicated by aspiration of significant quantities of fresh blood, which compromises cellular concentration, preservation and interpretation.

In an attempt to overcome this problem, an alternative fine needle sampling method developed in France[1-4] was further investigated. The technique, employing insertion of a fine needle into a lesion without attachment of a syringe, relies on the property of capillary tension in narrow channels. This physical principle states that a fluid (or semifluid substance) will ascend spontaneously into a narrow tube, in inverse proportion to the diameter of that tube (Figure 1). This method of nonaspiration fine needle cytology, which utilizes no active suction or aspiration, has been called "cytopuncture" by French authors.[1] This study tested the technique on 50 thyroid nodules, with simultaneously performed conventional FNAs serving as controls.

Materials and Methods

Over an eight-month period, 50 consecutive patients attending the Thyroid Clinic at Baragwanath Hospital with solitary or dominant solid thyroid nodules had their lesions studied by two sampling procedures: conventional FNA and the newly described nonaspiration needle technique. Both procedures employed 22-gauge needles with an outer diameter of 0.6 mm. Conventional FNA was performed using a 10-mL syringe in a syringe holder. The nonaspiration needle sample was obtained by inserting the needle, held between the thumb and forefinger of one hand,

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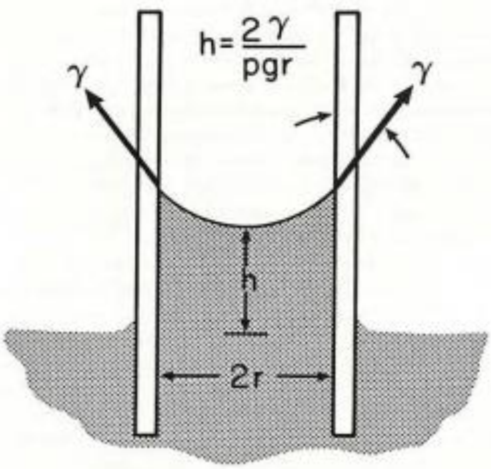

Figure 1
The principle of capillary tension. The ascent of fluid into a narrow channel is governed by the formula $h = 2\gamma/pgr$, where h is the height attained, γ is the fluid surface tension, p is the density of that fluid, g is the gravity and r is the radius of the tube.

into the mass and moving the needle rapidly in and out within the confines of the nodule, as with the conventional FNA method, but without attachment to a syringe or holder (Figure 2). Upon withdrawal of the needle, a syringe was then attached to enable expression of the needle contents onto glass slides. For each technique, as many smears were prepared as the amount of material dictated. Half of the slides were spray fixed using an alcohol fixative while the remainder were permitted to air dry. All sampling procedures were performed by one operator (J.E.C.S.).

Upon receipt in the laboratory, the alcohol-fixed and air-dried smears were stained with the Papanicolaou and Giemsa stains, respectively. All smears were interpreted by a single observer (G.L.), without knowledge of the technique employed for any particular set of slides. Apart from the diagnosis, comments were rendered on the quality of the slides. For this purpose, three qualitative categories were created. (1) Specimens were considered "unsuitable" for cytodiagnosis if they consisted mainly of blood or if cellular material was absent, making them inadequate for determination of benign or malignant changes. (2) Specimens were categorized as "diagnostic/adequate" when it was possible to render an opinion on the nature of the lesion sampled, but when the cellular material present was suboptimal due to poor cellularity, sample dilution, degenerative changes or specimen entrapment in blood clots. (3) Specimens were noted as "diagnostic/superior" if the cells or cell aggregates were concentrated, well preserved, unobscured by background blood and excellently displayed, with retention of such architectural structures as follicles, papillae and flat sheets. Specimens in this category were those from which textbook-quality illustrations could have been taken.

Results

In only one patient, who has failed to return for

Figure 2
The technique of nonaspiration fine needle cytology. The nodule to be sampled is fixed by the operator's left hand. The needle, acting as both probe and suction channel, is held between the thumb and index finger of the right hand and is advanced into the nodule.

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Table I *Performance of Aspiration and Nonaspiration Needle Cytology Techniques in Benign and Malignant Thyroid Nodules*

	Thyroid nodule			
	Benign lesions (n=36)		Neoplastic lesions (n=13)	
Results/ performance	Nonaspiration cytology	FNA cytology	Nonaspiration cytology	FNA cytology
Diagnostic superior	18	3	4	1
Diagnostic	16	29	9	10
Unsuitable	2	4	—	2

Table II *Performance of Aspiration and Nonaspiration Cytology in Total Series (50 Cases)*

	Technique			
	Nonaspiration cytology		FNA cytology	
Results/performance	No. of cases	%	No. of cases	%
Diagnostic superior	22	44*	4	8*
Diagnostic	25	50	39	78
Unsuitable	3	6	7	14

*P=.0033 (McNemar's test for significant change).

reevaluation, were both sampling methods productive of inadequate material, representing 2.0% of the total study group. The results of the FNA and nonaspiration procedures in 49 evaluable cases are summarized in Table I. Benign lesions, comprising 72% of the cases, were predominantly colloid or hyperplastic nodules (32 cases); the other 4 cases were Hashimoto's thyroiditis. Of the 13 neoplasms (26% of the total), 5 were papillary carcinomas, 6 were follicular neoplasms and 1 each was a medullary or anaplastic tumor. The numbers in each individual diagnostic category do not permit statistical analysis, but, for the entire benign group, significantly more "diagnostically superior" specimens (18 vs. 3) were obtained by the nonaspiration technique. A similar trend was evident in the neoplastic group, in which four samples obtained without aspiration were diagnostically superior, as compared with only one obtained by conventional aspiration. The unsuitable category included three nonaspiration specimens and seven aspiration specimens.

When benign and neoplastic lesions were analyzed together, statistically significant results emerged. As seen in Table II, which includes the single patient without satisfactory cytology, diagnostically superior material was obtained in 22 (44%) of the nonaspiration samples versus 4 (8%) of aspiration samples. The difference is significant at the level of P=.0033 by McNemar's test of significant change.

Discussion

When performed by a skillful aspirator and assessed by an experienced observer, material obtained by conventional FNA methods, often with fairly high suction applied, is usually evaluable, whether or not the cellular component is perfectly preserved and displayed. All practicing cytologists will, however, be aware of the problems posed by specimens of marginal quality and the ease with which inaccurate diagnoses can be rendered on such specimens. Few centers have followed the Karolinska model, in which the cytopathologist routinely performs the aspirations. In most cases, FNA samplings are performed by clinicians with varying skills and experiences with the technique. Because FNA has been promoted as a "simple" test, it is often relegated to junior members of staff, with far-from-perfect results.

Any innovation, therefore, that inherently produces samples of better quality is worthy of consideration. The nonaspiration technique tested in this series of thyroid lesions has been utilized in France but has not been widely publicized or promoted. The thyroid was selected for our initial clinical trial of this procedure since it is a vascular organ that notoriously produces heavily blood-stained aspirates. Furthermore, many diagnostic pitfalls exist in the interpretation of thyroid specimens,[3] making excellence of cellular material a prerequisite for diagnosis in both experienced and less-experienced laboratories. While the number of unsuitable specimens in this series was not statistically different with FNA or nonaspiration needle sampling, the quality of the material studied was significantly superior in the nonaspiration samples. Although the average of four slides (and range of two to six) made from nonaspiration samples was less than the average of six slides (and range of two to ten) made from FNA samples, the cellular material was more concentrated, less traumatized and less likely to be obscured by blood or distorted within blood clots in the nonaspiration smers. It would appear that the presence of blood in thyroid cytology samples cannot be entirely prevented; its effect on smear quality was, however, minimized by the spontaneous capillary action of the new technique, as opposed to the active, often high, suction pressures of conventional FNA procedures. Morphologic details were thus improved, resulting in easier interpretation and shorter screening times.

At the clinical level, it was noted that fewer patients complained of pain or discomfort with the nonaspiration technique, although this was not statistically computed. Furthermore, the technique was not only simpler for the operator, eliminating the need for a syringe holder, but also better enabled an enhanced appreciation of the consistency of the mass being sampled. The needle, held directly in the fingers, acted as a sensor, or sensitive probe, in this respect, providing tactile information not yielded when syringes and syringe holders were employed.

Had only conventional FNA cytology been employed in this series, seven (14%) of the diagnoses would not have been possible. The corresponding failure rate for nonaspiration cytology would have been 6% (three cases). These differences are not significant, but of importance is that one of the failures of the conventional method involved the single anaplastic carcinoma in the group. Utilization of both procedures reduced the failure rate to 2%. It is anticipated, therefore, that both methods will continue to be used in tandem until such time as future analysis of broadened experience shows clear sampling superiority of the needle-only method.

Initial scepticism concerning the ability of a nonaspiration technique to obtain diagnostic material from thyroid masses has been allayed. Doubts may persist with respect to more fibrous tumors of lower cellularity, for example, breast neoplasms, which evoke a fibrous response in the stroma. In this regard, small numbers of breast tumors and other palpable soft tissue masses have been sampled in a similarly comparative fashion. Initial results are very promising for the new technique, but are as yet inadequate for statistical analysis. Good results with the use of the technique to sample orbital and periorbital tumors have been reported by Zajdela et al[4] while Briffod et al[1] have used the technique in the follow-up of breast carcinoma cases. The latter paper refers to other studies in France that have utilized nonaspiration fine needle sampling.

There is no doubt that problems of nomenclature, more vast and more serious than those that have plagued conventional FNA, will supervene should this technique become more widely used. The term *nonaspiration fine needle cytology* employed here is descriptive, but is not fluent or memorable; nor does it lend itself easily to abbreviation. Common parlance in this center has tended toward "capillary suction technique." Alternate terminology, such as "cytopuncture," will undoubtedly be suggested. A strong plea is made that any suggested nomenclature, which should at least indicate the elimination of aspiration, should not include the term *biopsy*, which seems singularly inappropriate to this sampling technique.

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tively exclude carcinoma and therefore render biopsy unnecessary. Nonetheless, the ultrasonographic characteristics of a nodule may be of use in estimating cancer risk in patients in whom needle biopsy was inadequate and those who are hesitant to undergo or prefer to delay biopsy.

Thyroid ultrasonography has important limitations (18,19). The procedure is very operator dependent, and ultrasound reports often lack crucial information, such as the dimensions of nodules in all three planes (length, width, and depth), the location of a nodule in the thyroid lobe, comments about density or calcification, the appearance of the border of the nodule, and the presence of lymph nodes. Ultrasonography is usually performed as a diagnostic test, with no intent on the part of the ordering physician to have a biopsy done or no capability for biopsy at the ultrasound facility. Patients are better served if ultrasonography is done under conditions in which biopsy can be performed immediately if indicated. The operator must be interested in and knowledgeable about thyroid nodules, know what to look for, and be able to use the results to perform needle biopsy at the same time.

Computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) using 19-fluorodeoxyglucose have no established role in the evaluation of patients with thyroid nodules. These tests are listed in Table 73.3 only because in some patients thyroid nodules are discovered when the test was performed for other reasons. A single thyroid nodule identified on a PET scan may be a carcinoma, but some benign nodules and Hashimoto's thyroiditis take up substantial quantities of 19-fluorodeoxyglucose as well.

FINE-NEEDLE BIOPSY

Fine-needle biopsy, which yields single cells or clumps of cells, is the standard test to determine whether a thyroid nodule is benign or malignant, and the test also has other uses (Table 73.4). It is often called fine-needle aspiration biopsy, but the more general term will be used here because aspiration by negative pressure is not always used. The primary goal of fine-needle biopsy is to reduce the number of unnecessary thyroid operations, by identifying those nodules that are benign or at least unlikely to be malignant. For this purpose, fine-needle biopsy is far more accurate than any other available test or combination of tests. Before the procedure was introduced, about half of patients with palpable thyroid nodules were referred for operation, but only about one fourth of those who underwent surgery had thyroid carcinoma. Hence, many patients with benign nodules were subjected to the costs, morbidity, and, rarely, risk for mortality of surgery (20, 21).

The use of fine-needle biopsy can reduce the proportion of nodules for which surgery is advised to less than 20%; at the same time, the proportion of resected nodules that are malignant is 50% or even higher. When an adequate sample is obtained, the reliability of fine-needle biopsy for excluding carcinoma is 98% to 99%. The second main use of fine-needle biopsy is to help plan the extent of surgery. Large-needle biopsy, which provides cores or fragments of tissue for conventional histologic evaluation, is rarely used now. Details of the methods can be found elsewhere (22).

TABLE 73.4. USES OF THYROID NEEDLE BIOPSY

Common
Selection of therapy for a thyroid nodule
Surgery versus observation
Extent of surgery
Less common
Diagnosis of the cause of thyroid enlargement
Removal of fluid or blood from a cystic nodule
Injection of a sclerosing agent for a recurrent cyst
Ablation of a nodule by ethanol injection or laser coagulation
Postoperative evaluation of cervical lymph nodes
Diagnosis of infective thyroiditis

Selection of Patients

Fine-needle biopsy is the most appropriate test for almost all patients with thyroid nodules, the exception being patients suspected to have an autonomously functioning thyroid adenoma. Biopsy is usually advised if the maximal diameter of the nodule is 1 cm or higher (23,24), but some researchers suggest a threshold of 1.5 or 2.0 cm (25,26). This distinction depends in part on the method of ascertainment. Nodules smaller than 1.5 cm are often not palpable, whereas much smaller nodules can be detected by ultrasonography. Even if ultrasonography is performed, or the nodule is detected incidentally when an imaging study of the neck is done for another purpose, nodules smaller than 1 cm in diameter are often not biopsied. The rationales for this threshold are that smaller nodules are so common that it is impractical to refer all of them for biopsy, and the risk for missing a carcinoma that will have adverse health consequences is low. Many people have small thyroid carcinomas that never become evident during life and cause no health problems. The prevalence of thyroid carcinoma at autopsy is about 4% in the United States (25), where there are currently about 2.5 million deaths per year, fewer than 24,000 incident cases of thyroid carcinoma diagnosed per year, and fewer than 1,500 deaths from thyroid carcinoma per year (27). Furthermore, among patients with thyroid carcinoma, the outcome is strongly influenced by the size of the tumor at the time of diagnosis: the life expectancy of patients with a tumor that is smaller than 1 cm in diameter is the same as that of the general population (see section Radioiodine and Other Treatments and Outcomes in Chapter 70).

As noted above, incidental thyroid nodules may be detected by ultrasonography or other imaging procedures performed to evaluate neck structures other than the thyroid gland, or by thyroid ultrasonography done to evaluate neck pain, difficulty swallowing, or a known thyroid abnormality. Most of these nodules are too small to be detected by palpation. Others are located in the middle of a thyroid lobe (and do not distort its shape), or behind or below the lobe (28); have a soft consistency; or are hidden by subcutaneous fat, prominent neck muscles, or kyphosis of the cervical spine. These factors are independent of the biology of the nodule, and therefore it is likely that a nonpalpable nodule is a carcinoma as often as is a palpable nodule of the same size. The available data support this conclusion. Among patients with nonpalpable, incidentally discovered thyroid nodules who have undergone fine-needle biopsy—by necessity ultrasound-guided—4% to 10% have had thyroid carcinoma (3,29–32), very similar to the results in patients with palpable nodules. Therefore, the evaluation, including the size cut-off for biopsy, should be the same no matter how the nodule was detected.

Fine-needle biopsy is advisable in patients with a multinodular goiter who have a discrete hypofunctioning nodule within the goiter or a nodule of uncertain functional status that is growing, and patients with a partially cystic nodule after fluid has been removed. The prevalence of carcinoma in these nodules is similar to that in solitary solid nodules (3,6,7). Fine-needle biopsy is also indicated when a hypofunctioning area is seen in an otherwise hyperfunctioning nodule, although the great majority of these areas represent central degeneration within the nodule, not carcinoma. A few patients with thyrotoxicosis caused by Graves' disease also have hypofunctioning nodules, whether detected by palpation or scintiscan; these nodules should be biopsied because some are carcinomas. Patients treated with head and neck radiation for nonthyroid conditions, or accidentally exposed to radiation, who have thyroid nodules should be evaluated by biopsy like any other patient; biopsy results are as accurate in these patients as in patients without such a history (33,34).

Pregnant women found to have a thyroid nodule should undergo biopsy, like any other patient, for two reasons. First, the detection of the nodule raises concern about carcinoma that can usually be alleviated simply and quickly. Second, biopsy facilitates decisions regarding surgery in women whose nodules should be excised, including the options of surgery in the second trimester, as soon as possible after delivery, or at a time most consistent with plans for breast-feeding (35,36).

Fine-needle biopsy may not be as accurate in children as in adults, as discussed later.

Technique of Fine-Needle Biopsy

About two thirds of thyroid specialists in the United States currently perform fine-needle biopsy guided by palpation, whereas about two thirds in Europe use ultrasound guidance (37). The routine use of ultrasonography increases the expense of the procedure, usually requires advance scheduling, and requires an assistant. In a study from a single institution comparing the diagnostic accuracy of palpation-guided fine-needle biopsy of nearly 5,000 thyroid nodules with ultrasound-guided fine-needle biopsy of a similar number of nodules (38), the diagnostic accuracy was better when ultrasonography was used, but the differences between the two groups were small, and patients were not randomly allocated to each group. The biggest difference was in the frequency of biopsies in which the sample was inadequate; it was 3.5% for the ultrasound-guided biopsies versus 8.7% for the palpation-guided biopsies. The results were falsely negative (missed carcinoma) in 1% of nodules examined via biopsy with ultrasound guidance and in 2.3% of the nodules examined via biopsy with palpation guidance, although there were more nodules smaller than 1 cm in the ultrasound-guided biopsy group.

Palpation-guided fine-needle biopsy is satisfactory for nodules that are easily palpable. For nodules difficult to palpate, and of course those that are not palpable, the biopsy must be guided by ultrasonography. If palpation-guided biopsy yields an inadequate sample, an ultrasound-guided biopsy should be performed, especially if the nodule is relatively small. The alternate approach of doing only ultrasound-guided biopsies is favored by some physicians.

The actual procedure is performed with the patient supine, with a pillow under the shoulders to facilitate neck extension. The patient should be asked not to talk or swallow while the needle is in the neck. The skin is cleaned with alcohol, and the skin where the biopsy needle is to be inserted is infiltrated with 1 to 2 mL of 1% lidocaine (this may be omitted). The nodule is fixed by the fingers of one hand, and the needle in the other hand is inserted perpendicular to the anterior surface of the neck. Twenty-five-gauge, 1½-inch needles provide excellent specimens and are less likely than larger needles to cause bleeding that dilutes the specimens. The ease with which bleeding is induced depends on whether the nodule is dense and relatively avascular, or loosely organized and more vascular. Occasionally, 22- or 23-gauge needles may be needed to obtain adequate specimens from hard, fibrotic nodules.

The biopsy sample may be obtained without or with suction. In the nonsuction technique, the needle is gripped like a pencil, which facilitates precise needle placement for small nodules, and allows the needle to be moved both in and out over a few millimeters and rotated (39). This combined motion uses the bevel of the needle for cutting, which frees cells that flow into the needle by capillary action while the needle is held steady for about 10 seconds. Material can usually be seen entering the hub of the needle. Samples obtained in this way are less likely to be diluted with blood than samples obtained using suction. If the nonsuction method does not yield a satisfactory sample, or if cyst fluid requires removal, suction is used.

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