CHAPTER 1
INTRODUCTION

The chapter gives an overview of the research project and includes the background of the study, the problem statement and the importance of conducting a study of this kind. The aim and objectives are defined.

1.1 BACKGROUND OF THE STUDY

A STN is a discrete lesion within the thyroid gland that is palpably and or sonographically distinct from the surrounding thyroid parenchyma. Patients presenting with a palpable thyroid nodule are a common clinical dilemma and the optimum diagnostic strategy for patients with nodular thyroid disease remains a matter of debate. Thyroid nodules have an estimated prevalence of 2 to 6% by palpation in the general population and the figure may be much higher by ultrasonography (USG). The majority of thyroid nodules are benign with cancers accounting for about 5%. Thyroid cancers are classified as well differentiated (follicular, papillary or mixed) or poorly differentiated (anaplastic). Differentiated thyroid cancers (DTCs) have excellent prognosis with good long-term survival when appropriate and timely intervention strategies are offered to these patients. Distinguishing malignant nodules from benign nodule avoids expensive evaluation and treatment of otherwise benign disease.

FNA cytology is a well-established technique and is considered the gold standard in the pre-surgical diagnostic evaluation of thyroid nodules. This approach is sufficient in most cases except for follicular neoplasia because distinction between benign and
malignant follicular neoplasia can only be made by examining the presence of capsular or vascular invasion on a formal biopsy specimen. Unfortunately, FTC is more common than PTC in developing countries - like at our centre. This necessitates an operative procedure and tissue sample before the final diagnosis of a follicular lesion is concluded. At times, the choice between direct total thyroidectomy and the more risky two-stage surgery in the event of malignancy found at postoperative histology is a problem, especially when facilities for frozen section are not readily available. Furthermore, even when available, intraoperative frozen section may not be conclusive because its role in the detection of minimally invasive FTC or a follicular variant of PTC is limited. A second operative procedure is risky because of the likelihood of damage to vital structures (recurrent laryngeal nerve and parathyroid glands) due to post-operative fibrosis from the initial surgery. For this reason, there is need for an appropriate, non-invasive diagnostic approach that will enable differentiation of follicular adenoma from FTC preoperatively to minimise the problem of second stage surgery for a malignant thyroid nodule.

Evaluation of STN can be quite challenging to clinicians as clinical, biochemical and even FNA cytologic examination can be inconclusive. Most cases require further assessment by means of functional imaging with scintigraphy or anatomical imaging in the form of USG or computed tomography (CT).

Scintigraphy has a long and established role in the evaluation of STN. Scintigraphic imaging using the radiopharmaceuticals $^{99m}$TcO$_4^-$, $^{123}$Iodine ($^{123}$I) or $^{131}$Iodine ($^{131}$I) is able to assess the functionality of a thyroid nodule - a hypofunctioning (or cold) nodule
is a nodule that has decreased uptake on thyroid scintigraphy when compared to adjacent thyroid tissue. The probability of malignancy in a hypofunctioning STN is approximately 16% \( ^8 \) – the diagnostic accuracy is too low to reliably confirm or exclude malignancy, hence the need for second-line imaging.

Thyroid nodules have also been evaluated using \( ^{99m} \text{Tc-MIBI} \). \(^9,10,11 \) This agent was initially introduced for cardiac imaging. However early biodistribution studies demonstrated accumulation in normal tissue-dense organs (such as the thyroid and parathyroid glands, bones, muscle, liver spleen, lung and breast); as well as in neoplastic disorders. \(^9,10 \) MIBI is a lipophilic cationic complex that accumulates and is retained in areas of increased mitochondrial activity. Malignant cells have accelerated mitochondrial activity that fuels their aberrant growth and metabolism, and will therefore retain MIBI. \(^10 \) This property makes MIBI a tumour-seeking agent.

USG is non-invasive, relatively cheap, readily available and free of ionising radiation. It is often the first line imaging modality for the initial work-up of clinically detected thyroid nodule. \(^12 \) Numerous grey scale sonographic features have been found to be highly suggestive of thyroid malignancy, including microcalcifications, marked hypoechochogenicity, irregular or microlobulated margins, and the lesion assume a taller than wider shape configuration. \(^3-5,13 \) In addition, colour and power Doppler methods allow easy identification of suspected thyroid nodules by displaying a characteristic vascular pattern, and thus results in better assessment of thyroid nodular vascularisation. The most common pattern of vascularity associated with malignancy is intranodular vascularity. \(^3-5,13 \) Despite these characteristic features, USG is underutilised
and often may still be unhelpful in differentiating benign from malignant thyroid nodules, probably because of some overlap of sonographic features that exist between benign and malignant disease,\textsuperscript{13,14} or partly because of operator dependency of the USG techniques.

1.2 RATIONALE OF THE STUDY

A STN is a common problem encountered in clinical practice. Differentiation between a malignant and a benign process on clinical grounds alone is difficult, and therefore often requires the use of an invasive biopsy in the form of FNA. In general, the accuracy of FNA is good, however FNA has difficulty in diagnosing FTC – this is problematic in our loco-regional setting where follicular neoplasia is more common. Thus there exists the possibility of missing early FTC or patients undergoing operative surgical procedure for otherwise benign disease. Therefore there is need for an effective concerted imaging approach for thyroid nodule evaluation to sharpen diagnostic accuracy.

The evaluation of thyroid nodules by means of Nuclear Medicine imaging using \textsuperscript{99m}Tc-MIBI and radiological imaging using USG has been studied independently.\textsuperscript{2,8-11,14,67-77} However, the diagnostic utility / comparison of the two imaging approaches in combination has not been widely utilised.

1.3 RESEARCH QUESTION

Will the combination of anatomical (USG) and physiological (\textsuperscript{99m}Tc-MIBI scintigraphy) imaging improve differentiation between benign and malignant STN?
1.4 RESEARCH AIM AND OBJECTIVES

The overall aim of the study was to determine if combined and $^{99m}$Tc-MIBI scintigraphy and USG imaging will improve preoperative differentiation of a benign from a malignant STN.

The objectives of the study were as follows:

I. To evaluate patients with STNs using $^{99m}$Tc-MIBI scintigraphy.

II. To evaluate patients with STNs using USG.

III. To compare the sensitivity, specificity, positive and negative predictive values of the two respective imaging modalities against histopathological findings.
CHAPTER 2
THEORETICAL FRAMEWORK AND LITERATURE REVIEW

This chapter gives an overview of theoretical framework of thyroid disease, with an emphasis on nodular pathology of the thyroid gland. The thyroid gland is discussed in terms of its anatomy and physiology, thyroid nodule classification, clinical and imaging evaluation, and management. This chapter highlights imaging of a STN particularly with $^{99m}$Tc-MIBI and USG. This research intends to achieve or add to the existing literature repertoire.

2.1 THE THYROID GLAND

The name “thyroid” is derived from the Greek word *thyreous* meaning “oblong shield”; it is attributed to Thomas Warton in the 17th century. The thyroid gland is the largest endocrine gland and weighs about 15 to 20 grams in an adult.$^{16,17}$

2.1.1 Development and anatomy

The thyroid gland develops from first and second pharyngeal pouches at about 24 days (4 weeks) of gestation and descends to a point just inferior to the thyroid cartilage, one lobe on each side of the trachea. Ectopic tissue can therefore be found anywhere from the foramen caecum at the base of the tongue to the myocardium.$^{17}$

The thyroid gland is located anterior to the trachea and below the thyroid cartilage (Figure 2.1). The two lobes of the thyroid measure 4 to 5cm each from superior to
inferior poles and are 1.5 to 2cm wide. The isthmus connects the two lobes, and shows considerable anatomic variability.\textsuperscript{17} The thyroid is made up of numerous follicles filled with colloid and lined by cuboidal epithelial cells. The colloid’s major element is thyroglobulin, a glycoprotein containing the thyroid hormones thyroxine (T3) and triiodothyronine (T4).\textsuperscript{17,18} The major epithelial cells present in the thyroid gland are the follicular cells, which are responsible for synthesis of thyroid hormones and the parafollicular cells (C-cells), which secrete calcitonin.\textsuperscript{19} Thyroid neoplasia can arise from these different types of cells or from non-epithelial stromal elements, forming the basis of histologic classification of tumours.

The gland is highly vascular and is majorly supplied by the (i) inferior thyroid artery – the largest branch of thyrocervical trunk of the subclavian artery, and (ii) the superior thyroid artery – a branch of the external carotid artery. In 10\% of population the thyroidea ima artery, which arises from aortic arch, brachiocephalic trunk or internal carotid artery, supplies the isthmus. The gland is drained by three pairs of veins - superior and middle thyroid veins, which drain into the internal jugular. The inferior thyroid veins drain into the brachiocephalic veins.\textsuperscript{20}

The lymphatic vessels from the thyroid gland drain to pretracheal, paratracheal and deep cervical lymph nodes. Some drain directly to the brachiocephalic vessels or into the thoracic duct.\textsuperscript{20}
2.1.2 Normal thyroid gland on scintigraphy and ultrasonography

For better understanding of the appearances of a thyroid nodule or any other abnormality, it is important to know the normal appearance of how the thyroid gland appears on these two imaging modalities.

Scintigraphically, the normal thyroid gland appears symmetrical and the lateral borders of lobes are straight to convex (Figure 2.2). The tracer is distributed uniformly within the gland. It is also seen normally in salivary glands and in capillary network of the neck tissue (also called ‘blood pool’). Thyroid nodules are described according to the degree of “uptake” that they demonstrate. Most nodules are hypofunctioning relative to
surrounding tissue and will accordingly have decreased uptake on thyroid scintigraphy when compared to adjacent thyroid tissue.

Figure 2.2: A planar anterior image of the thyroid demonstrating a normal thyroid scintigram (image obtained from Medical Imaging–orvosi kepalkotas).

Sonogaraphically, a normal thyroid lobe on transverse section has a triangular shape, with three edges (Figure 2.3). The structure is granulated (ground glass appearance) and the echogenicity is similar to that of the parotid glands, and higher compared to that of the strap muscles. Most STN nodules are discrete and hypoechogenic when compared to the adjacent strap muscle. The thyroid arteries may be localised on colour
or power Doppler. A low resistance flow is demonstrated on spectral Doppler in these visceral arteries. A peak systolic velocity in the intra-thyroid arteries is on the range of 15 to 30 cm per second and is the highest velocity found in any superficial organ. Exclusive peri-nodular vascularity or absent flow is associated with benign nodule whereas marked intranodular vascularity with chaotic arrangement is associated with malignant nodule.²,¹³

Figure 2.3: Axial section of the normal thyroid sonogram. Showing the two thyroid lobes (arrows) connected by the isthmus (image obtained from 2013—AIUM practice guideline—Thyroid and Parathyroid Ultrasound).
2.1.3 Thyroid gland physiology

Iodine is necessary for the synthesis of thyroid hormones. Iodine ingested orally is rapidly reduced to iodide in the upper small intestine and finally absorbed into the bloodstream in about the same manner as chlorides.\textsuperscript{17,18} In order to form normal quantities of thyroxine, about 50 milligrams of ingested iodine in the form of iodides are required each year, or about 1 mg / week.\textsuperscript{18} To prevent iodine deficiency, common table salt is iodized with about 1 part sodium iodide to every 100,000 parts sodium chloride.\textsuperscript{18}

Normally, most of the iodides are rapidly excreted by the kidneys, but only about one fifth is selectively removed from the circulating blood by the cells of the thyroid gland and used for synthesis of the thyroid hormones.\textsuperscript{18}

The iodine uptake, thyroid hormone synthesis and release are summarized as follows\textsuperscript{17,18}:

- **Iodine transport**: the thyroid follicular cells traps iodide by means of a high energy sodium iodide “thyroid pump” that concentrates iodide intracellularly at 25 to 500 times the plasma concentration.

- **Oxidation**: once the iodide is trapped in the thyroid cell it is converted to an oxidised or neutral form of iodine by thyroid peroxidase at the follicular cell colloid interface.

- **Organification**: iodine couples with tyrosine and forms mono- or di-iodinated tyrosines (MIT or DIT).

- **Coupling**: Two DIT form T4 or one DIT and one MIT form T3.

- **Storage**: T3 and T4 migrate to the colloid space in the middle of the thyroid follicle.
- **Release**: T3 and T4 are secreted by reversing the storage process and reversal of the migration through the follicular cell membrane.

Thyroid stimulating hormone (TSH) initiates iodine uptake and organification, as well as release of thyroid hormones through hydrolysis of thyroglobulin. Thyroglobulin does not normally enter the bloodstream except during disease states such as thyroiditis and thyroid cancer.\(^{16}\)

To maintain normal thyroid function, the hypothalamus and the pituitary gland both influence thyroid status. The process is referred to as the “hypothalamic-pituitary-thyroid axis”; this link between the three is what regulates proper thyroid hormone synthesis and release (Figure 2.4).

Most thyroid nodules (relative to surrounding normal tissue) lose their ability to trap and organify iodine (hypofunctioning) – they therefore appear cold on \(^{99m}\)TcO\(_4^-\), or \(^{123}\)I thyroid scintigraphy.
2.2 THE THYROID NODULE

2.2.1 Aetiopathogenesis

The occurrence of a thyroid nodule is determined by a complex interplay between environmental, genetic and dietary factors.\(^{24}\)
Iodine deficiency is believed to be an important predisposing factor to the development of nodular goitre or follicular neoplasm.\textsuperscript{24-26} This is supported by the fact that addition of iodide supplement to the diet has resulted in a decreased incidence of follicular cancer and a relative increase in the incidence of papillary thyroid cancer.\textsuperscript{26,27} Overstimulation by TSH due to chronic iodine deficiency has been considered to play a vital role in tumourigenesis.\textsuperscript{27}

Radiation and genetics are other important causal factors. Exposure to ionising radiation in childhood and adolescence increases the risk of both nodules and thyroid carcinoma.\textsuperscript{28} Radiation causes various forms of gene mutations that can result in development of neoplasia.\textsuperscript{26} There are familial thyroid cancers that are associated with genetic abnormalities; this is best understood for familial medullary thyroid cancer (MTC) and multiple endocrine neoplasia (MEN 2) syndromes.\textsuperscript{17,28,29} There is also growing evidence to suggest that some differentiated thyroid cancers of follicular cell origin are familial in nature.\textsuperscript{29-33}

Non-neoplastic nodules are usually the result of compensatory glandular hyperplasia due to partial thyroidectomy or may occur spontaneously; rarely, hemi-agenesis of the thyroid gland may present as hyperplasia of the existing lobe, simulating a thyroid nodule.\textsuperscript{34} Non-neoplastic thyroid disorders, such as Hashimoto's thyroiditis or subacute thyroiditis may appear as thyroid lumps which are not true nodules but just the expression of the underlying thyroid disease.\textsuperscript{34}
2.2.2 Epidemiology / prevalence

The prevalence of STN varies with different geographic locations and methods of detection. Thyroid nodules have an estimated prevalence of 2 to 6% by palpation in general population\textsuperscript{1,2} with a female to male ratio of 5:1 in iodine-deficient areas.\textsuperscript{35} Discrepancy exists between the true prevalence of thyroid nodules and that obtained by physical examination. With the increasing availability of sensitive imaging techniques, the nodules are emerging with greater clarity and demanding our attention. Between 30% and 50% of adults have one or more nodules in the thyroid gland on ultrasonic examination\textsuperscript{36}; and 20% to 48% of patients with single palpable thyroid nodule are found to have additional nodules on USG investigation.\textsuperscript{37} The frequency of thyroid nodules increases throughout life; a 5 to 10% lifetime risk exists for developing a palpable thyroid nodule.\textsuperscript{35}

The risk of malignancy in patient with STN is reported to be 5% and malignancy is about 3 times more common in females.\textsuperscript{3,36} This gender difference is found in almost all countries – with one exception being the pre-pubertal age group, in whom the incidence in boys and girls is almost the same.\textsuperscript{36} Although the age of patients with thyroid cancers may slightly differ from one histologic type to the other, the average age of the patient with differentiated thyroid cancer is between 35 to 40 years.\textsuperscript{36} The peak incidence at about 40 years is different from most malignancies that are more prevalent with advancing age.\textsuperscript{36}
Studies have shown that focal thyroid nodules detected by $^{99m}$Tc-MIBI scintigraphy $^8$ or $^{18}$F-fluorodeoxyglucose positron emission tomography ($^{18}$F-FDG PET),$^{38,39}$ have a high risk of malignancy. The American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association (AACE/AME/ETA) Thyroid Nodule Guidelines$^{25}$ recommend that such lesions should undergo focused sonographic examination followed by FNA cytology.

2.2.3 Classification of thyroid nodules

As defined previously, a STN is a single discrete nodule within the thyroid gland that is detected either by palpation and or by means ultrasonographic examination as distinct from the surrounding thyroid parenchyma. STN can be broadly classified into benign and malignant nodules.$^{19,25,34,35,40-42}$

**Benign thyroid nodules**

Majority of the nodules are benign and can be classified as:

- Adenomas
- Teratomas
- Colloid nodules
- Cysts
- Infectious nodules
- Lymphocytic or granulomatous nodules
- Hyperplastic nodules
- Thyroiditis and
- Congenital abnormalities such as thyroglossal duct cyst

The true thyroid adenomas are discrete and encapsulated (a feature that differentiates them from hyperplastic nodule which is not capsulated) and are further classified as follows:9,25,34,35,40,43:

- Papillary adenoma
- Follicular adenoma
- Hurthle cell adenoma

The follicular adenomas can be subdivided according to the size of follicles into colloid, embryonal and foetal varieties.34,35,40,43 Follicular adenomas are the most common and most likely to mimic the normal function of the thyroid tissue and this often present as a STN.40 They are usually solitary, smaller than 3 cm and become palpable when they reach 5 to 10 mm in size.44 Follicular adenomas tend to grow slowly, to remain unchanged for years at a time, and become symptomatic only late and rarely.40,44 The vast majority of follicular adenomas (more than 95%) are hypofunctional on radioiodine scintigraphy. About 5% of these nodules may be hyperfunctional, concentrating iodine very avidly, which may suppress the function in the remainder of the thyroid.40,44 They may occasionally produce thyrotoxicosis (toxic adenoma), a finding that is also rare in nodule less than 3cm in size.40,45,46 Hyperfunctioning adenomas are a frequent cause of T3 toxicosis and are amenable to ablation by surgery or 131I treatment.46,47
Malignant thyroid nodules

Malignant thyroid nodules can be classified as\textsuperscript{19,25,34,35,40-42}:

I. Differentiated:

A. Papillary carcinomas:
   
   i. Pure papillary
   
   ii. Mixed papillary
   
   iii. Follicular carcinoma (variant including tall cell, follicular, oxyphil, solid).

PTC is the most common of thyroid malignancies, accounting for 80 to 90\%\textsuperscript{19,35,40} of all thyroid cancers world-wide but in iodine-deficient areas like ours, FTC is more common\textsuperscript{6,7}.

B. Follicular carcinomas
   
   i. Malignant adenoma
   
   ii. Hurthle cell carcinoma
   
   iii. Oxyphil carcinoma
   
   iv. Clear cell carcinoma and
   
   v. Insular carcinoma

II. Medullary thyroid carcinoma

MTC is not a tumour of follicular cell origin but it arises from the parafollicular cells (or “C” cells) of the thyroid gland.
III. Undifferentiated:

A. Small cell (to be differentiated from lymphoma)
B. Giant cell
C. Carcinosarcoma

IV. Miscellaneous:

A. Lymphoma, sarcoma
B. Squamous cell epidermoid carcinoma
C. Fibrosarcoma
D. Mucoepithelial carcinoma
E. Metastatic tumour

Because the thyroid gland has a rich blood supply, it is a common site for metastatic dissemination from primary tumours elsewhere, e.g., malignant melanoma and carcinoma of lung, breast, kidney, and oesophagus; and thyroid metastases may be the only presenting feature of primary disease.\textsuperscript{48} Thyroid lymphoma is the most common tumour occurring in women between the ages of 55 to 75 years, who have chronic lymphocytic thyroiditis with positive serum anti-thyroglobulin (anti-Tg) antibodies.\textsuperscript{40}

2.2.4 Clinical and laboratory evaluation

The evaluation of STN is done by taking a detailed medical history, conducting general physical examination, determining thyroid hormonal profile (thyroid function tests), performing imaging and invasive procedures such as FNA cytology. So, for evaluation, three questions must be asked and have to be answered:-
a. Is the nodule benign or malignant?

b. Is the nodule causing pressure symptoms on adjacent structures of the neck?

c. Is the nodule secreting excess thyroid hormone?

Thus, to answer these questions, it is imperative that patients presenting with thyroid nodule be thoroughly evaluated by way of meticulous clinical history and examination and focused laboratory and imaging investigations.

**History and examination**

Evaluation of STN includes history of the thyroid mass, past medical history, family and social history. Past history of radiation exposure to the neck is very essential. A meticulous clinical review of all systems including a careful, complete head and neck examination is indicated. Symptoms such as neck pain, dyspnoea, hoarseness of voice, stridor and dysphasia usually suggest thyroid malignancy, but are not diagnostic. Past medical history or family history of phaeochromocytoma, hypertension, chronic diarrhoea and constipation, hyperparathyroidism, and episodes of irritability or nervousness suggest the possibility of familial MEN type 2 or 2b syndrome.25,33,35,40-42,49,50

**Points in favour of benign pathology:**

a. Family history of Hashimoto’s thyroiditis

b. Symptoms of hypo-or hyperthyroidism

c. Pain or tenderness associated with the nodule
d. Surface of nodule being soft, smooth, and mobile on clinical examination

e. Multinodular goitre without a dominant nodule

f. Female gender

**Points in favour of malignancy:**

a. Young patients (< 20 years) or old (>70 years)

b. Male gender

c. History of external neck radiation during childhood or adolescent

d. Recent change in voice (hoarseness)

e. Difficulty in swallowing (dysphasia)

f. Past or family history of thyroid carcinoma

g. Firm consistency of nodule, its irregular shape, its fixation to underlying or overlying tissues

h. Suspicious ipsilateral cervical lymphadenopathy.

A longstanding nodule that is not enlarging is likely to be benign. Benign nodular lesions are more common in females than males. The reverse is true for malignant lesions. Hence, nodular lesions raise more suspicion of carcinoma in men than in women.

**Metabolic profile and other markers**

These include thyroid function tests, serum thyroglobulin assay, serum calcitonin level, serum anti-thyroid peroxidase (anti-TPO) & anti-Tg antibodies, serum carcinoembryonic antigen (CEA), and molecular markers.\(^\text{25,34,35,40,42,50}\)
A. Thyroid function tests

These are useful in the assessment of the functionality of nodule(s). Most patients with thyroid cancer are euthyroid - except for few cases of thyrotoxicosis, usually in patients with disseminated follicular cancer. Benign disorders like autonomously functioning adenoma or Hashimoto’s thyroiditis are more often associated with hypothyroidism, although patients may be toxic in the acute phase of Hashimoto’s (Hashitoxicosis) as well as in the case of larger adenomas. There is a strong association between Hashimoto’s thyroiditis and primary thyroid lymphoma.51

B. Serum thyroglobulin level

It cannot differentiate a benign from a malignant thyroid nodule unless the level is markedly increased, in which case metastatic thyroid cancer should be suspected.25, 34, 40 Because serum thyroglobulin may be increased in many thyroid diseases, routine measurement for initial evaluation of thyroid nodules is not recommended.37

However, in patients undergoing surgery for malignancy, testing of serum thyroglobulin may be considered so as not to overlook a false-negative serum thyroglobulin value due to decreased thyroglobulin immunoreactivity or heterophilic antibodies.25 Another important indication for thyroglobulin assay is for monitoring patients with nodules being followed non-operatively and in those patients who have undergone total thyroidectomy for thyroid cancer, excluding medullary thyroid cancer.25, 35, 40 Ideally, serum thyroglobulin should be measured under TSH stimulation.
C. Serum calcitonin level

It is increased in patients with MTC or MEN-2 and correlates well with tumour burden. Several reports recommend routine measurement of calcitonin in patients with a thyroid nodule.

D. Serum anti-thyroid peroxidase antibody and anti-thyroglobulin antibody levels

These are helpful in chronic autoimmune thyroiditis especially if serum TSH is elevated. Often in Hashimoto’s thyroiditis, the size and consistency of the thyroid gland resembles a solitary nodule or bilateral nodules. In this situation, the presence of Anti-TPO and Anti-Tg antibodies are helpful for diagnosis. But, evidence for Hashimoto’s disease does not eliminate the possibility of cancer; moreover there is strong association between primary thyroid lymphoma and Hashimoto’s thyroiditis or chronic lymphocytic thyroiditis.

E. Serum carcinoembryonic antigen level

The CEA has been proven to be a useful tumour marker in patients with MTC and it is elevated in more than 50% of patients with MTC.

F. Molecular markers

Many molecular and cytohistochemical markers have been evaluated to separate benign from malignant cytologically suspicious tumours. These include BRAF, PAX8,
Galectin-3, HBME, and RET/PTC, some of which are available commercially. However, the reported accuracy and predictive value of these tests are variable and at times discordant. FNA smears staining positive for galectin-3 are more likely malignant than those that do not.\textsuperscript{42,50}

### 2.2.5 Imaging work-up: morphologic or radiologic imaging

The radiologic imaging includes USG, CT and magnetic resonance imaging (MRI).

#### I. Ultrasonography

USG is a simple method that has become the basis of each morphological examination of the thyroid gland. It allows the assessment of the size of the gland, detection of nodules and evaluation of the echogenicity of the whole thyroid gland and nodules (hypo-, hyper-, or isoechogetic, echofree structure or calcification etc). In addition, enlarged lymph nodes in the neck may be detected and localized.\textsuperscript{15,37,40} Even a 1 to 2 mm size non-palpable nodule within the thyroid tissue can be detected with high resolution USG.\textsuperscript{37, 40} This inherent property of USG makes it an excellent imaging method to guide biopsy in non-palpable thyroid nodule. Solid or mixed lesions are consistent with tumour, but may be either benign or malignant. Positive predictive criteria of malignancy include solid marked hypoechoic nodules, presence of fine punctate microcalcifications, irregular shape or microlobulations, taller than wide shape, absence of halo, and marked intranodular vascularity.\textsuperscript{3-5,13} The main limitation of this technique is considerable overlap between benign and malignant thyroid nodules and operator dependency of the technique.\textsuperscript{13,14}
II. Computed tomography and magnetic resonance imaging

CT and MRI have a more limited role in the initial evaluation of STN. But their main indications include suspected tracheal involvement either by invasion or compression, extension into the mediastinum (retrosternal lesions), or detection of residual or recurrent disease.\textsuperscript{40}

2.2.6 Imaging work-up: functional or radionuclide imaging

Functional thyroid imaging may be performed using a number of radiopharmaceuticals, including \(^{99m}\text{TcO}_4^\text{-}\), \(^{123/131}\text{I}\), \(^{99m}\text{Tc-MIBI}\) and thallium-201 (\(^{201}\text{Tl}\)). Other tracers such as \(^{123/131}\text{I-MIBG}\) or octreotide and \(^{18}\text{F-FDG PET}\) have also been used for thyroid imaging.

I. \(^{99m}\text{Tc-pertechnetate}\) or \(^{123/131}\text{Iodine}\)

The mechanism of localisation of \(^{99m}\text{TcO}_4^\text{-}\) and \(^{123/131}\text{I}\) is by means of a high energy sodium iodide thyroid pump similar to normal dietary iodine as described above. \(^{99m}\text{TcO}_4^\text{-}\) is an iodine structural analogue that is taken up by the thyroid follicular cells in a similar mechanism as \(^{123/131}\text{I}\). In contrast to \(^{123/131}\text{I}\), it is not organified or coupled into the thyroid hormones, and it washes out of the gland faster - necessitating the need to perform \(^{99m}\text{TcO}_4^\text{-}\) scintigraphy within 20 minutes of tracer injection.\textsuperscript{17,18}

Scintigraphy with \(^{99m}\text{TcO}_4^\text{-}\) or \(^{123}\text{I}\) can determine the functionality of a nodule / thyroid gland and correlate the location of palpable nodule with nodules seen with USG. Depending upon the degree of uptake of the radioactive isotope, thyroid nodules are further classified into cold, decreased or no uptake compared to the rest of thyroid
gland; warm, uptake greater than the rest of thyroid tissue; and hot - increased uptake with suppression of the remainder of the thyroid gland (Figure 2.5). The majority of STNs will appear cold. These cold nodules have an approximately 5% to 15% chance of malignancy. Of the 5% nodules shown to be “hot” on scintigraphy, about 1% are malignant. Therefore, one cannot rely solely on $^{99m}$TcO$_4^-$ scintigraphy to differentiate between benign and malignant thyroid nodules with great accuracy. In an attempt to improve the specificity of the study, some authors recommend adding a vascular or perfusion imaging phase. The rationale being that malignant nodules do not obey normal homeostatic control mechanisms that regulate growth and metabolism – in order to fuel this accelerated growth and metabolism, they tend to have enhanced blood flow and will therefore appear more vascular than surrounding tissue. Thus, a nodule that is vascular on the perfusion phase but cold on the uptake phase (a so called “perfusion-uptake mismatch”) is more likely to be malignant. Adding a vascular phase results in a modest improvement of specificity for malignancy to around 40%. Whereas a nodule that is non-vascular and cold (“perfusion-uptake match”) is more likely to be benign - however there still remains an overlap with some highly cellular benign lesions (such as adenomas) also demonstrating a flow-uptake mismatch.
**Figure 2.5**: Illustration of functional types of thyroid nodules as seen on scintigraphy: (A) cold, (B) warm, (C) Hot.

**II. $^{99m}$Tc-MIBI scintigraphy**

Because of the low specificity of $^{99m}$TcO$_4^-$ as mentioned above, there exists the need for second line scintigraphic imaging to further assess STN that exhibit a perfusion-uptake mismatch. $^{99m}$Tc-MIBI scintigraphy has successfully been utilised to fulfil this role. Malignant cells have high mitochondrial activity - MIBI being a lipophilic cationic complex, it passively diffuses across the cell membrane into the cell. Inside the cell, it has high affinity for mitochondria resulting in its retention. Mitochondrial retention is driven by negative transmembrane potential. In those nodules that exhibit flow-uptake mismatch, $^{99m}$Tc-MIBI scintigraphy increases the sensitivity to 77%. $^{99m}$Tc-MIBI scintigraphy is likely to be more sensitive and accurate than FNA cytology in the assessment of follicular neoplasia. The positive predictive features of $^{99m}$Tc-MIBI scintigraphy are increased accumulation of tracer on both early and delayed images. The likelihood of malignancy increases with the intensity of tracer accumulation as well. One advantage of this technique is that patients need not discontinue thyroid hormone replacement before imaging.
III. Positron emission tomography (PET)

FDG-PET is the most widely used tracer in oncologic evaluation due to the increased glucose utilisation of the malignant cells. However, the role of FDG-PET in combination with CT (PET-CT) will play in thyroid cancer remains in evolution. As indicated, early studies have shown that there can be ‘flip-flop’ in uptake patterns in thyroid cancers, with them being first iodine positive, FDG negative, and with progression becoming FDG positive, iodine negative. At this stage it is considered to have dedifferentiated in to a high grade. For this reason, FDG-PET is not indicated in the initial work-up of the STN or well differentiated thyroid cancers.$^{55,56}$

Other alternative PET tracers that have been used for thyroid imaging is positron emitting radioiodine ($^{124}$I). $^{124}$I-PET has been used in imaging thyroid cancer patients for dosimetry and for lesion detection; then may provide insights into individual tumour’s response to therapy. $^{124}$I-PET can serve as a highly robust predictor for $^{131}$I therapy and in addition can be diagnostically useful for thyroid cancer staging. However, due to high cost of production this tracer is only available in few hospitals worldwide.$^{56-58}$

The role of $^{68}$Gallium(Ga)-DOTA-conjugated peptide PET is well established in the visualisation of tumours originating from neural crest cells such as MTC and can be used to determine somatostatin-receptor status prior to peptide receptor radionuclide therapy (PRRNT). Being a tumour of neural crest origin and is known to variably express somatostatin receptor that makes visualisation with $^{68}$Ga-DOTA-conjugated peptide PET possible.$^{59}$
2.2.7 FNA cytology

FNA cytology has a central role in decisions that affect the management of patients with thyroid nodules. It is the most specific investigation to differentiate between benign and malignant nodules. The diagnostic accuracy of FNA is close to 98%, with rates of false positives and false negatives that are less than 2%. But its shortcoming is missing of the malignant area, especially FTC, diagnosis of which relies on presence of vascular or capsular invasion – this is not possible with FNA cytology. FNA cytology specimens of follicular neoplasms and Hurthle cells are usually interpreted as indeterminate or suspicious. This has resulted in low FNA cytology accuracy rates of about 40% for FTC, which is significantly lower than that quoted for $^{99m}$Tc-MIBI.

Therefore, in areas of endemically higher FTC rather than PTC, the addition of a $^{99m}$Tc-MIBI scintigraphy to the diagnostic work up of a patient with a STN adds value. The results can aid the referring physician in determining which patients require an expedited biopsy. Thus, it needs expertise and experience. According to the cytologic findings, nodules can be classified as benign (about 70%), suspicious (about 10%), malignant (about 5%) or non-diagnostic (about 15%).

2.2.8 Evaluation and treatment of a thyroid nodule

The discovery of a thyroid nodule is naturally a source of concern to the patient. Very few of these nodules will require surgery, but once this choice has been excluded, the physician is faced with a variety of non-surgical options, and choosing the optimal approach can be a difficult task.
Comprehensive medical history and thorough physical examination is followed by US evaluation for additional unsuspected nodule and record sonographic appearances to assess risk for malignancy. Thyroid scintigraphy and thyroid function tests are done to determine the nodule functionality. FNA cytology being the next step and depending on cytologic findings, various management approaches are available including observation, levothyroxine suppression therapy, surgery, radioiodine ablation (RAI) and non-surgical minimally invasive procedures (percutaneous ethanol injection [PEI], percutaneous laser ablation [PLA] and radiofrequency [RFA] ablation). \(^\text{42,61-64}\)

A. Observation / levothyroxine (or T4) suppression therapy

Patients with proven benign solitary thyroid nodules may undergo observation or levothyroxine suppression therapy as the initial treatment modality. Administration of T4 is aimed at shrinking nodule size, arresting further nodule growth, and preventing the appearance of new nodules. Routine use of T4 suppressive therapy in nodular thyroid disease is not recommended because of its potential long-term adverse effects, such as osteoporosis and cardiac arrhythmias.

Levothyroxine suppression therapy is more likely to be beneficial in (i) small, recently diagnosed nodules in younger patients living in an iodine deficient area and (ii) in nodules with colloid features on cytology with no evidence of functional autonomy.

A growth of a thyroid nodule during levothyroxine therapy is a strong indication for surgery. \(^\text{42,61}\)
B. Surgery or thyroidectomy

Surgical options include lobectomy plus isthmectomy for a benign nodule and near-total or total thyroidectomy for malignant disease.

Cytologically suspicious nodules can be treated with thyroid lobectomy plus isthmectomy or total thyroidectomy; the latter is preferred if the patient is hyperthyroid, has a history of head and neck radiation, or has bilateral nodules. If facility for frozen section is available it should be performed at the time of surgery to help guide surgical decision making, but may be of limited use in distinguishing benign from malignant follicular lesions.

C. Radioiodine ablation

The aim of RAI treatment is the ablation of autonomously functioning nodule (either toxic or non-toxic). The effectiveness of RAI in reduction in size of the thyroid gland is widely recognised and, for this purpose, it has also been used for the treatment of non-autonomous thyroid nodules.

RAI is preferred over thyroidectomy for small, non-toxic goitres without suspicion for thyroid malignancy, in patients previously treated with surgery, or in those at risk for surgical intervention.

RAI is not the treatment of choice if compressive symptoms are present, or in larger nodules requiring high doses of $^{131}$I (which may be resistant to treatment).\textsuperscript{42,61}
D. Non-surgical minimally invasive procedures

Procedures such as PEI, PLA and RFA have been used in the treatment of benign thyroid nodule with no suspicion for malignancy.61-64

Percutaneous ethanol injection: A clinically significant decrease in size of after PEI has been reported in patients who have benign, solitary cold solid nodules. The procedure appears to be more effective than T4 therapy in decreasing nodule volume and in relieving local pressure symptoms. PEI is an effective alternative to surgery in the treatment of complex nodules that are predominantly cystic. Aspiration of thyroid cysts decreases the volume, but recurrences are common, and surgery is often required to remove large, relapsing lesions. A reduction of greater than 50% of the baseline size is obtained in nearly 90% of cases treated with PEI.42,61,62

Percutaneous laser ablation: Laser is an acronym for Light Amplified Stimulated Emission of Radiation. Laser technology directs collimated, monochromatic, coherent, and powerful light energy to a delineated area of tissue in a predictable, precise, and controlled manner. Tissue is destroyed primarily by energy absorption leading to coagulation of micro-vessels and ischaemic injury. PLA is a minimally invasive procedure that is proposed as an alternative to surgery for thyroid nodules causing local symptoms or cosmetic concerns.42,61,63

Radiofrequency ablation: Basically, the term RFA refers to an alternating electric current oscillating between 200 and 1200 kHz. Application of radiofrequency power to tissue agitates tissue ions as they attempt to follow the changes in direction of the
alternating current. Such agitation creates frictional heat around the electrode with resultant tissue injury. RFA is a relatively novel procedure that is used widely for inoperable liver tumours but under evaluation as a non-surgical therapeutic modality for the ablation of benign and malignant thyroid lesions. This may play a potential role in a safe and effective alternative to surgery for treating benign, non-functioning, or autonomously functioning nodules as well as for recurrent thyroid cancers.\textsuperscript{42,61,63,64}

\section*{2.2.9 Preventive measures}

Prophylactic iodine supplementation programmes designed to correct these deficiencies can reduce the prevalence of nodules. There is assumption that individual iodine supplementation can also be used to decrease the volume of existing thyroid nodules.\textsuperscript{61}

ALARA principle states that “as low as reasonably achievable” (ALARA) radiation exposure should be maintained – so also radiation exposure to the head & neck region should be minimised. Both iodine deficiency and head & neck irradiation are associated with increased risk for the development of thyroid neoplasm.

\section*{2.3 PREVIOUS STUDIES ON THYROID NODULE EVALUATION}

Thyroid nodule is a topic in clinical medicine that presents a diagnostic dilemma because virtually any thyroid disease can manifest itself as thyroid nodule - the major issue in management of STN is the diagnostic work-up and the extent of thyroidectomy.\textsuperscript{65,66} Of particular concern is in iodine deficient area like ours in which FTC is more prevalent and the distinction of malignancy from benign follicular adenoma
is often difficult on FNA cytology in the absence of capsular or vascular invasion. This means that most early FTCs are often missed at FNA and this has prompted the need for this research.

Most STN are benign but the significance of STN lies in the increased risk of malignancy compared to the other causes of thyroid swelling. The challenge in clinical setting is identification of the small population of patients that are likely to have a malignant lesion from the vast majority with benign nodules, to avert unnecessary surgery in about 90% of patients with STN. Thyroid gland pathologies have been widely researched in terms of preoperative diagnosis including the role of FNA cytology, functional imaging using radionuclide scintigraphy and USG. FNA cytology is generally regarded as gold standard for diagnosis of thyroid lesions may result in up to 20% (or more) inconclusive cytologies due to overlap of benign and malignant cytologic features – especially in follicular neoplasia.

Before the introduction of $^{99m}$Tc-MIBI as a potential tumour seeking agent, radionuclide $^{201}$Tl was used in the evaluation of thyroid nodules using the single photon emission computed tomography (SPECT) technique. $^{99m}$Tc-MIBI displays some advantages when compared with $^{201}$Tl scintigraphy: (1) $^{99m}$Tc photon energy of 140keV which is particularly suitable for scintigraphic imaging with modern gamma cameras (less attenuation and scatter); (2) a shorter half-life and more favourable biodistribution kinetics, with a lower radiation exposure for the patient, and thus room for a higher injected dose; and (3) higher tumour-to-background ratios. On the basis of these considerations, $^{99m}$Tc-MIBI provides better quality diagnostic images than $^{201}$Tl.
Moreover, MIBI can be kept in stock and is easily labelled with $^{99m}\text{TcO}_4^-$, unlike $^{201}\text{Tl}$ which must be prepared offsite and delivered to the facility on the day of the study. It is, therefore, valid to say that $^{99m}\text{Tc}$-MIBI is more suitable agent for pre-operative evaluation of a hypofunctioning thyroid nodule.

The interesting findings with $^{99m}\text{Tc}$-MIBI scintigraphy in the evaluation of thyroid nodules are its high negative predictive value (NPV) and high sensitivity.\textsuperscript{11,69-71} Wale \textit{et al},\textsuperscript{69} in a recent retrospective review of local data combined with a meta-analysis of published literature, demonstrated that $^{99m}\text{Tc}$-MIBI scintigraphy showed a very high NPV of 97% and the sensitivity of 96%. Theissen \textit{et al}\textsuperscript{71} also reported similarly high NPV of 97% compared to 94% obtained with FNA cytology. A previously published meta-analysis study (448 patients) which correlated $^{99m}\text{Tc}$-MIBI uptake with histology in which $^{99m}\text{Tc}$-MIBI scintigraphy was used in patients with hypofunctioning thyroid nodules. This publication found that the NPV of $^{99m}\text{Tc}$-MIBI scan was 100% in patients with differentiated and medullary thyroid cancers.\textsuperscript{11}

Renewed interest in the use of $^{99m}\text{Tc}$-MIBI for the evaluation of thyroid nodules has been shown in recently published papers.\textsuperscript{69,70,72-74} Because of the high NPV, high sensitivity and cost effectiveness of $^{99m}\text{Tc}$-MIBI scintigraphy in the evaluation of thyroid nodules, Verburg \textit{et al}\textsuperscript{72} advocate that $^{99m}\text{Tc}$-MIBI should be registered for scintigraphic evaluation of thyroid nodules and that a thyroid nodule $^{99m}\text{Tc}$-MIBI scintigraphy protocol be adopted as routine evaluation of hypofunctioning thyroid nodules. And that, if successfully registered, it could return Nuclear Medicine to the forefront of thyroid nodule diagnosis. The work by Wale \textit{et al}\textsuperscript{69} further shows signs of an interesting trend:
the evaluation of a thyroid nodule by scintigraphy alone. Nearly a fifth of patients included in the authors’ cohort did not undergo FNA cytology, and management instead relied on (negative) $^{99m}$Tc-MIBI scintigraphy alone.

Sathekge *et al* in a prospective study of 71 patients with a hypofunctioning thyroid nodule evaluated using $^{99m}$TcO$_4^-$ and $^{99m}$Tc-MIBI and compared against FNA cytology and histological examination. Specificity of $^{99m}$Tc-MIBI, $^{99m}$TcO$_4^-$, and FNA were 77%, 40%, and 90%, respectively. Another study$^{10}$ evaluated $^{99m}$Tc-MIBI avidity on a 4-point scale - score 2 and score 3 or in combination as predictor of malignancy. In this study, score 2 and score 3 were defined as uptake equal to the normal thyroid tissue and uptake greater than normal thyroid tissue respectively. Sensitivities of score 3 and score 2+3 $^{99m}$Tc-MIBI uptake patterns were 83% and 100% respectively. The score 3 $^{99m}$Tc-MIBI uptake pattern had a specificity of 100% and a positive predictive value (PPV) of 100% with respect to differentiating benign and malignant thyroid nodules, whereas a specificity of 72% and a PPV of 43% were observed for detecting differentiated cancers. This means that the more intense the $^{99m}$Tc-MIBI uptake is on early and / or delayed scans, the greater the chances of a nodule being malignant.$^9$

However, one of the limitations of $^{99m}$Tc-MIBI is its low PPV.$^{69,70,74}$ Like FNA cytology, $^{99m}$Tc-MIBI scintigraphy cannot reliably differentiate follicular adenoma from FC because it also accumulates variably in follicular adenoma and Hurthle cell adenoma. Hurtado-Lopez *et al* demonstrated that about two-third of their cohorts with histologically confirmed benign thyroid lesions also had positive $^{99m}$Tc-MIBI scintigraphic findings.
Many studies have investigated whether the ultrasonographic characteristics of thyroid nodules, grey scale and colour / power Doppler USG are useful indicators for differentiating malignant from benign lesions of the thyroid. These show a wide range of variations.75-77 Kim et al75 evaluated 82 patients with thyroid nodule classified as positive for malignancy, 46 were malignant. Of 73 lesions classified as negative by USG, 3 were malignant. The sensitivity, specificity, PPV, NPV and accuracy were respectively 93.8%, 66%, 56.1%, 95.9% and 74.8%. A different study by Koike et al77 found that the sensitivity and specificity of preoperative USG diagnosis of thyroid nodules were 81.8% and 90.6% respectively. However, the sensitivity of USG for differentiating follicular adenoma from FTC, a common form of thyroid neoplasia in this environment, was disappointingly low (18.2%).

It is pertinent to note that most of the studies dwelt on comparing 99mTc-MIBI scintigraphy with FNA cytology or comparing USG with FNA cytology. There are only few studies that compared scintigraphy and USG in evaluation of nodular thyroid disease. In addition this Author has not come across a study that compared or assessed 99mTc-MIBI scintigraphy with USG. The closest attempt is by Demirel et al78 which compared the radionuclide thyroid angiography (RTA), 99mTc-MIBI scintigraphy and Power Doppler USG (PDUS) in the differential diagnosis of solitary cold thyroid nodules. Significantly in this study, grey scale USG was not included. In their study, they found out that PDUS was the most sensitive; RTA was the most accurate and specific in differentiating benign from malignant thyroid nodules, whereas 99mTc-MIBI performance was also good but did not show obvious superiority over other imaging modalities.
Sensitivity, specificity, accuracy, PPV and NPV were respectively 89%, 100%, 97%, 100% and 97% for RTA; 100%, 76%, 81%, 53% and 100% for PDUS; and 67%, 91%, 86%, 67% and 91% for $^{99m}$Tc-MIBI scintigraphy.

Erdem et al $^{79}$ compared $^{99m}$Tc-tetrofosmin (lipophilic myocardial perfusion imaging agent similar to $^{99m}$Tc-MIB) with colour Doppler USG. They established that the sensitivity, specificity, NPV and PPV of $^{99m}$Tc-tetrafosmin scintigraphy and colour Doppler USG to be 100% and 80%, 85% and 80%, 60% and 50% and 100% and 94% respectively. In this study again, the authors compared only the Doppler USG feature with $^{99m}$Tc-tetrofosmin scintigraphy. A prospective study of 68 patients with thyroid nodules evaluated using $^{99m}$TcO$_4^-$ and USG by Cox et al $^{80}$ concluded that both $^{99m}$TcO$_4^-$ scintigraphy and USG do not accurately differentiate between benign and malignant conditions of the thyroid and that their routine use in the investigation of thyroid nodules should be abandoned. Again significantly in this study only compared $^{99m}$TcO$_4^-$ scintigraphy but not $^{99m}$Tc-MIBI with USG. Also a prospective comparison of $^{99m}$TcO4$^-$ and USG was made by Kaur et al $^{65}$ The findings were as follow: $^{99m}$TcO$_4^-$ was 100% sensitive and 23% specific, and USG was 71.4% sensitive and 77.7% specific.

In the extensive search of literature by this Author the following observations were made:

I. Thyroid nodules have been extensively studied either by means of $^{99m}$Tc-MIBI scintigraphy alone or using USG alone. Other studies evaluated thyroid nodules
using $^{99m}$TcO$_4^-$ (but not $^{99m}$Tc-MIBI) scintigraphy with US and compared with FNA cytology.

II. Thyroid nodules have also been evaluated using $^{99m}$TcO$_4^-$, $^{99m}$Tc-MIBI scintigraphy and PDUS and compared against FNA cytology and histopathologic examination.

III. However, to the best of my knowledge I have not encountered any study that compared $^{99m}$Tc-MIBI scintigraphy and USG (both grey scale and Doppler USG).

I conclude the review of this literature by stating that: $^{99m}$Tc-MIBI is relatively available and cost effective, involves a simple imaging protocol, and provides objective semi-quantitative information. It has been proven to be very helpful in differential diagnosis of thyroid nodules particularly due to its high NPV and sensitivity especially in the setting of inconclusive / non-diagnostic cytology. USG on the other hand is non-invasive, relatively cheap, and is free of ionising radiation. In addition, it is the best first line imaging modality that displays thyroid anatomy and that characterise thyroid nodule succinctly. Yet these two promising imaging modalities have not been explored or studied in combination in the pre-operative evaluation of nodular thyroid lesions.

The purpose of this study is set to determine the combined role of these two imaging modalities.
CHAPTER 3
MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter refers to the systematic, theoretical analysis of the methods applied to this study. It includes a description of study design, the research settings and study population, the procedures for sample enrolment & sample size, data collection methods, ethical consideration and validity & reliability of the research methods. The chapter also describes the instruments used, as well as the data analysis procedures.

3.2 STUDY DESIGN

This was a prospective evaluation of patients who presented with a STN, using $^{99m}$Tc-MIBI and USG, and compared against histopathological examination. Patients who consented to participate in the study were enrolled based on the inclusion. All patients had $^{99m}$TcO$_4^-$ and $^{99m}$Tc-MIBI thyroid scintigraphy and followed up with USG and final histopathological examination.

The study spanned over the period of one year between March 2013 and April 2014. The data were collated, checked on quality, and entered into the database for subsequent analysis and writing of the manuscript.
3.3 STUDY SITE AND POPULATION

The study was conducted at the departments of Nuclear Medicine and Radiology of Dr George Mukhari Academic Hospital (DGMAH).

The study population comprised of all patients with a thyroid nodule referred to the department of Nuclear Medicine for thyroid scintigraphy. All patients fulfilled criteria for inclusion in the study as mentioned below.

**Inclusion criteria:** patients with clinically palpable or sonographically detected thyroid nodule and who were clinically and biochemically euthyroid.

**Exclusion criteria:** patients with evidence of thyroid hyperfunction (clinical and biochemical), prior surgery, pregnant patients or breast feeding mothers who could not withhold breast milk for 24 hours after the study.

3.4 SAMPLING AND SAMPLE SIZE

A total 42 patients with thyroid nodules were enrolled into this prospective study. This sample size was determined based on the assumptions that the prevalence of palpable STN of 2 to 6% in general population and taking the prevalence to be 3% at DGMAH and at 95% confidence level. Sample size was thus calculated using Taylor’s formula:

\[ n = z^2 p (1-p)/d^2. \]

Where;

\( n = \) the desired sample size (mean)
\( z \) = the standard normal deviation usually set at 1.96 which corresponds to 95 percent confidence level.

\( p \) = proportion of population with thyroid nodule = 5% (5/100) = 0.05

\( d \) = degree of accuracy desired, usually set at 0.05.

\[
\begin{align*}
  n &= z^2 p (1-p)/d^2 \\
  &= (1.96)^2 (0.03) (1-0.03)/ (0.05)^2 = 45.
\end{align*}
\]

3.5 METHODS OF DATA COLLECTION

All patients referred to the department of Nuclear Medicine for evaluation of the STN had thyroid examination to appraise site, size, number and consistency of the nodule. Demographic data such as age and gender of patients was routinely recorded. Blood samples for thyroid function test were taken.

3.5.1 \(^{99m}\text{TcO}_4^-\) and \(^{99m}\text{Tc-MIBI}\) thyroid scintigraphy protocols

Both \(^{99m}\text{TcO}_4^-\) and Tc-MIBI thyroid scans were performed with a gamma camera (E.CAM; Siemens Medical Solution USA, Inc.) equipped with a 6-mm aperture pinhole and a low-energy, parallel-hole, high resolution collimators. Patients were imaged in the supine position with the neck extended. The gamma camera was centered over the thyroid, a 6-cm gap being maintained between the pinhole aperture and the thyroid. Both dual phase \(^{99m}\text{TcO}_4^-\) and \(^{99m}\text{Tc-MIBI}\) thyroid scintigraphies were performed according to the department of Nuclear Medicine, University of Limpopo / DGMAH protocol.
At the Department of Nuclear Medicine of DGMAH, all patients that present for thyroid evaluation routinely undergo scintigraphic dual phase assessment with $^{99m}$TcO$_4^-$. The angiographic phase of 60 frames of 1 second followed by 1 frame of 60 seconds (pool images) to complete a dynamic phase using a 64 x 64 matrix immediately post intravenous injection of 185MBq of $^{99m}$TcO$_4^-$ was first performed. This was followed by uptake images at 20 minutes post injection in multiple projections (anterior, obliques etc.) – (the uptake phase). $^{99m}$TcO$_4^-$ scintigraphy determined the functional nature of the thyroid nodule and gland function. A nodule that demonstrated no uptake or reduced uptake when compared to the surrounding thyroid tissue was considered to be “cold”. Cold nodules that were vascular were deemed to exhibit a “perfusion - uptake mismatch”. Such nodules were considered suspicious for malignancy (Figure 3.1). Cold nodules that were avascular (“perfusion – uptake match” pattern) were regarded as benign (Figure 3.2). However, regardless of the uptake pattern, all cold nodules were further evaluated using $^{99m}$Tc-MIBI scintigraphy.

Figure 3.1: demonstrates increased uniform flow but decreased uptake in the left lobe of the thyroid (perfusion / uptake mismatch).
Fig 3.2: demonstrates decreased perfusion and decreased uptake in the lower pole of the left lobe of the thyroid (perfusion / uptake mismatch).

**99mTc-MIBI scintigraphy**

A week after $^{99m}$TcO$_4^-$ scintigraphy, dual phase $^{99m}$Tc-MIBI imaging was performed in a 256 x 256 matrix post intravenous injection of 740MBq of the radiopharmaceutical. Images were acquired in the anterior projection, with the patient lying supine and the neck hyperextended, at 20 minutes (“early”) and 120 minutes (“delayed”) post injection.

**Assessment criteria:** the $^{99m}$Tc-MIBI images were qualitatively assessed by two independent Nuclear Medicine Physicians who were blinded to the clinical, US and cytology findings. The images were analysed on a 4-point score:

- $0 =$ no significant uptake
- $1 =$ uptake greater than the background activity but less normal thyroid tissue
- $2 =$ uptake equal to normal thyroid tissue
- $3 =$ uptake greater than normal thyroid tissue with retention on delayed images (Figure 3.3).
Score 3 was regarded as positive for malignancy while scores 0 to 2 were considered benign.\textsuperscript{8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig33.jpg}
\caption{Fig 3.3: demonstrates increase MIBI uptake that is greater than paranodular tissue / contralateral lobe and is retained on the delayed 2h image}
\end{figure}

\subsection*{3.5.2 Ultrasonography protocol}

Grey scale and colour, PDUS was performed by two board-certified Radiologists using broad band 5 to 10 MHz linear array transducer (Siemens Acuson X 300; Siemens Medical Solutions, Mountain View, USA). Patients were scanned in supine position with neck extended supported by a foam pad or pallor placed behind the neck. Ultrasonic gel thinly applied to the thyroid region and scanned in sagittal and transaxial plane. Nodule size and all the USG features that suggest malignant or benign nodule were documented.
Assessment criteria: USG characteristics that favoured malignancy were defined as microcalcifications (Figure 3.4), marked intranodular vascularity pattern on colour Doppler (type 3) [Figure 3.5], a shape that is taller than it is wider (Figure 3.6), an irregular or microlobulated margin and marked hypoechogenicity. If one or more of these features were present, the nodule was classified as positive for malignancy. If a nodule had none of these features, it was classified as negative or benign.

Figure 3.4: demonstrates multiple intranodular microcalcifications (left) and irregularity of the outline absent halo (right). Both features are suggestive of malignancy.

Figure 3.5: demonstrates type 3 intranodular vascularity (left) suggestive of malignancy and perinodular vascularity pattern (right) a feature of benign nodule.
Both scintigraphic and USG evaluation were performed without any knowledge of cytologic findings. All patients underwent FNA cytology, which was mostly done after the scintigraphic or USG evaluation.

### 3.5.3 FNA cytology / histopathology

All patients underwent FNA cytology, which were generally performed after USG and scintigraphic evaluation. All patients also underwent thyroidectomy or lobectomy and subsequent histopathologic evaluation. The histologic criteria were as follows:

- **PTC** = papillary configuration and classical nuclear changes,
- **FTC** = capsular and / or vascular invasion,
- **MTC** = groups of polygonal cells surrounded by amyloid
- Anaplastic carcinoma = undifferentiated cells
- Follicular adenoma = lesions composed of small follicles without capsular or vascular invasion
- Colloid goitre = follicles varying in sizes and shapes distended by colloid
• Hurthle cell adenoma = cells with abundant granular cytoplasm without capsular invasion.

Results of radionuclide scintigraphy and USG were compared against FNA cytology and histopathological examination.

3.6 DATA ANALYSIS

University of Limpopo (MEDUNSA) statistician assisted with analyses of the data (Appendix 1).

The data obtained from the structured data sheet was constituted in an Excel spreadsheet and subsequently entered into a statistical programme for analysis. Statistical analysis was performed using Statistical Analysis System (SAS), Release 9.2, running under Microsoft windows on a personal computer. The results were presented in the form of chart and tables. The sensitivity, specificity, accuracy, and positive and negative predictive values of each imaging methods were calculated using 2 x 2 contingency tables.

3.7 ETHICAL CONSIDERATIONS

3.7.1 Approval for the study

The research protocol was approved by the Medunsa Research and Ethics Committee (MREC) of the University of Limpopo (Medunsa campus) – Project Number MREC/M/20/2013: PG. Approval to conduct the study was also obtained from the
Hospital Clinical Management of DGMAH. The certificates of approval are attached as Appendix 2 and Appendix 3 respectively. Permission to use USG facility was also sought from the Head of Radiology Department of DGMAH (Appendix 4).

The data were collected using a structured data sheet (Appendix 5) and written consent was obtained from each patient after informing them regarding the study, in the language of their choice as far as possible. See attached consent form in Appendix 6.

3.7.2 Hazard to the patients and other ethical issues

The radiation exposure to the thyroid during $^{99m}$TcO$_4^-$ and $^{99m}$Tc-MIBI scintigraphy is minimal and confers no long term risk to the patient. USG is free of ionizing radiation and generally there is no risk associated with its application. No personal identifiers such as the name, or address of patients or doctors were recorded to ensure confidentiality and anonymity. For this reason patients were given research numbers e.g. NMR 001, NMR 002, NMR 003 and so on.

3.8 VALIDITY AND RELIABILITY

Scintigraphic and USG interpretation were performed by qualified Nuclear Medicine Physicians and Radiologists respectively without prior knowledge of FNA cytological findings. To ensure consistency, interpretation of scintigraphic images and USG were done by same specialists on all patients that participated in the study.
### 3.9 RESEARCH TIMELINE

**Table 3.1:** Research Timeline

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>TIME(months/weeks)</th>
<th>DURATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Proposal writing</td>
<td>3 months</td>
<td>July 2012 – September 2012</td>
</tr>
<tr>
<td>2 Obtained SREC provisional approval</td>
<td>1 months</td>
<td>September 2012 - October 2012</td>
</tr>
<tr>
<td>3 Obtained MREC approval</td>
<td>3 months</td>
<td>December 2012 – March 2013</td>
</tr>
<tr>
<td>4 Data collection</td>
<td>13 months</td>
<td>March 2013 – April 2014</td>
</tr>
<tr>
<td>5 Data analysis</td>
<td>2 weeks</td>
<td>April 2014 – May 2014</td>
</tr>
<tr>
<td>6 Writing up</td>
<td>2 months</td>
<td>May 2014 – June 2014</td>
</tr>
</tbody>
</table>
CHAPTER 4
RESULTS AND INTERPRETATION

4.1 INTRODUCTION

In this chapter the results and interpretation of findings are presented. The chapter is presented in the following order: demographic information of the patients, final histopathologic findings, correlation of various techniques with histopathologic findings - FNA cytology, $^{99m}$Tc-MIBI scintigraphy and USG. The comparison of MIBI and USG and combined (MIBI + USG) performance versus stand-alone MIBI or USG are also presented.

The sensitivity, specificity, PPV, NPV and accuracy of these diagnostic approaches were also determined from two by two (2 x 2) contingency tables.

4.2 PATIENTS’ DEMOGRAPHIC INFORMATION

A total of 42 patients (39 females and 3 males) diagnosed as having solitary hypofunctioning thyroid nodule on conventional thyroid scintigraphy were included in this study. Their age ranged between 19 to 73 years (mean age 44.76). Majority (53.4%) of the patients were between the ages of 31 to 50 years (Figure 4.1)
4.3 NODULES AND FINAL HISTOPATHOLOGIC FINDINGS

The size ranges of the nodules evaluated were between 1.5 to 10cm (mean size, 4.63cm) with 50% of these nodules fall between 3 and 5cm in size. Majority (67%) of the nodules were present in the right lobe of the thyroid (Figure 4.2).
The nodules were classified according to histologic findings (Figure 4.3). Of the 42 patients evaluated, 15/42 (35.7%) malignant and 27/42 (64.3%) benign lesion. The data confirmed that 8/15 (53%) patients had FTC and 7/15 (47%) had PTC (4 follicular variants and 3 classical papillary).

Of the benign lesions, 17/27 (63%) had colloid goitres; 6/27 (22.2%) had nodular goitres; 2/27 (7.4%) had follicular adenoma; and 2/27 (7.4%) had Hurthle cell adenoma.

Overall, patients with colloid goitres constituted the majority 17/42 (40%), confirming that it is the most prevalent of the nodular thyroid lesions; 8/42 (19%) patients had FTC and 7/42 (17%) patients had PTC respectively; nodular goitres were seen in 6/42 (14%) of
patients; 2/42 (5%) of the patients had follicular adenoma and 2/42 (5%) had Hurthle cell adenoma.

![Pie chart showing frequency of different histologic types of thyroid nodules, n = 42](image.png)

Figure 4.3: Showing frequency of different histologic types of thyroid nodules.

**4.4 CORRELATION OF THE VARIOUS DIAGNOSTIC MODALITIES WITH HISTOLOGY**

The results are summarised in Table 4.1.

**4.4.1 FNA cytology**

As summarised in Table 4.1, FNA cytology results were classified as benign, malignant, follicular neoplasia and inconclusive cytology.
On FNA cytology 22/23 (95.7%) of patients who had cytologic diagnoses of benign lesions were subsequently confirmed to be benign on final histopathologic examination however, with one false negative.

All of the 5/5 (100%) patients who had cytologic findings of malignancy were confirmed malignant on histology.

Eleven cases were reported as follicular neoplasia; 5/11 (45.5%) had benign and 6/11 (54.5%) had malignant nodules on histopathologic examination.

Three patients had inconclusive cytology, however all the 3 were proven to be malignant.

The result also shows that FNA was positive in only 5/15 (33%) of malignant lesions overall. However, FNA was positive in 4/7 (57%) of 7 PTCs and in only 1/8 (12.5%) of FTCs. This very low detection rate of FTC supports the existing literature on the intricacy of FNA cytology in the diagnosis of FTCs which is a common type of thyroid carcinoma in our population / institution.

Correct diagnosis of the 2/2 Hurthle cell adenoma was made on FNA cytology. The 2/2 follicular adenomas were reported as follicular neoplasia. FNA achieved a correct diagnosis of 16/17 (94%) of the colloid goitres – 1/17 (6%) was reported as follicular neoplasia. Nodular goitres had a correct diagnosis in 4/6 (67%), while 2/6 (33%) were reported as follicular neoplasia.
Table 4.1: Summary of $^{99m}$Tc-MIBI scintigraphy, USG and FNA findings

<table>
<thead>
<tr>
<th>Histology</th>
<th>No.</th>
<th>$^{99m}$Tc-MIBI</th>
<th>USG</th>
<th>FNA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular Ca</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Papillary Ca</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Hurthle adenoma</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Colloid goitre</td>
<td>17</td>
<td>2</td>
<td>15</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Nodular goitre</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>23</td>
<td>19</td>
<td>19</td>
<td>23</td>
</tr>
</tbody>
</table>

Ca., carcinoma; No., number; Pos., positive for malignancy; Neg., negative for malignancy; Ben., benign; Mal., malignant; FN, follicular neoplasm; Inc., inconclusive cytology.

4.4.2 $^{99m}$TcO$_4^-$ scintigraphy

All patient evaluated had hypofunctioning thyroid nodule on $^{99m}$TcO$_4^-$ scintigraphy. This was not part of the study however; $^{99m}$TcO$_4^-$ scintigraphy was included in this study to determine the functionality of the nodules and determine which patient would undergo further evaluation with $^{99m}$Tc-MIBI scintigraphy. Moreover, dual phase $^{99m}$TcO$_4^-$ thyroid scintigraphy is a routine departmental protocol for thyroid scintigraphy at our centre.

Features suggestive malignancy on $^{99m}$TcO$_4^-$ scintigraphy is increased activity on the perfusion a corresponding cold area on uptake image. Features of benign lesion on $^{99m}$TcO$_4^-$ scintigraphy are decreased or no activity on perfusion with similar corresponding pattern on uptake images. Examples will be shown in the subsequent heading (under $^{99m}$Tc-MIBI features).
4.4.3 $^{99m}$Tc-MIBI scintigraphy

The results in Table 4.1 show that $23/42 (54.8\%)$ patients had nodules positive for malignancy on $^{99m}$Tc-MIBI scan. Out of these, $15/23 (65.2\%)$ had histologic confirmation of malignancy. The $^{99m}$Tc-MIBI scan was positive (score 3) in all $15/15 (100\%)$ patients with histologically proven carcinomas. However, false positive was observed in $8/27 (29.6\%)$ of patients with benign nodules. Table 4.2 shows correlation of $^{99m}$Tc-MIBI with histopathological findings.

**Table 4.2: Correlation of $^{99m}$Tc-MIBI with histopathological diagnosis**

<table>
<thead>
<tr>
<th>MIBI Scan Findings</th>
<th>Number (%) according to histologic diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Benign</td>
<td>19(70.4)</td>
<td>0(00.0)</td>
</tr>
<tr>
<td>Malignant</td>
<td>8(29.6)</td>
<td>15(100)</td>
</tr>
<tr>
<td>Total</td>
<td>27(100.0)</td>
<td>15(100.0)</td>
</tr>
</tbody>
</table>

Note: All findings are not statistically significant ($p$ value $> 0.05$, using McNemar’s Test)

All the $19/19 (100\%)$ patients that had negative (benign) $^{99m}$Tc-MIBI lesions were proven histologically to be benign – affirming its reported high NPV. Figure 4.4 displays $^{99m}$Tc-MIBI retention patterns on the early 20-minute and 2-hour delayed images (score 3) and was also positive (perfusion / uptake mismatch) on $^{99m}$TcO$_4$ scan – a pattern that is regarded as positive for malignancy. The lesion was histologically proven to be a follicular carcinoma.
Figure 4.4 scans showing 65 year old female patient with $^{99m}$TcO$_4$ uniform activity in the perfusion image and a cold area on the uptake image in the mid to lower zones of the left lobe (mismatch, positive for malignancy)-upper images. The lesion was also positive (score 3) on MIBI. The histologic diagnosis was follicular carcinoma.

It is also observed that the likelihood of malignancy increased with intensity of $^{99m}$Tc-MIBI on the early and delayed images and retention on the delayed scan. However, potentials for false positive $^{99m}$Tc-MIBI scan exist with adenomas. Figure 4.5 shows example of falsely positive scans (both $^{99m}$Tc-MIBI and $^{99m}$TcO$_4$) – histologic diagnosis was benign. This explains that benign lesions such as adenoma may show variable degrees of $^{99m}$Tc-MIBI avidity and / or retention.
Figure 4.5 scans showing 57 year old female patient with $^{99m}$TcO$_4$ uniform activity in the perfusion image and a cold area on the uptake image in the mid to lower zones of the right lobe (mismatch, positive for malignancy)-upper images. The lesion was also positive (score 3) on MIBI. The histologic diagnosis was benign.

4.4.4 Ultrasonography

All patients in the study had STN on clinical examination. USG revealed additional nodule(s) in 16/42(38%) of cases; and 4/16(25%) of these had carcinomas. Most of the nodules were solid with only one patient had mixture of solid and cystic.

The patients’ nodular thyroid were classified as malignant if they had one or more of these USG criteria including microcalcifications, intranodular vascularity type 3, irregular / microlobulated margin, marked hypoechogenicity, nodule that is taller than wider shape, and incomplete halo sign.
There were 19/42 (45.2%) patients with USG positive nodules, 13/19 (68.4%) of whom had proven carcinomas. USG was positive in 13/15 (87%) of patients with carcinomas, and falsely negative in 2/15 (13%) patients. Out of 23 patients with negative nodules on USG, 21/23 (91%) were truly benign. The results also revealed that 6/27 (22.2%) of benign lesions were falsely positive on USG.

Table 4.3 shows the correlation between USG and histopathological diagnoses.

**Table 4.3: Correlation of USG with histopathological diagnoses**

<table>
<thead>
<tr>
<th>USG Findings</th>
<th>Number (%) according to histologic diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Benign</td>
<td>21(77.8)</td>
<td>2(13.3)</td>
</tr>
<tr>
<td>Malignant</td>
<td>6(22.2)</td>
<td>13(86.7)</td>
</tr>
<tr>
<td>Total</td>
<td>27(100)</td>
<td>15(100)</td>
</tr>
</tbody>
</table>

Note: All findings are not statistically significant (p value >0.05, using McNemar’s Test)

The most frequently encountered and a reliable sonographic criterion for malignancy is intranodular microcalcifications. It is observed in this study that “snowstorm” pattern of microcalcifications both singly or in combination with other USG criteria was the most frequently encountered and were present in 8/15 (53%) of all carcinomas overall – in 6/7 (86%) of PTCs and in 2/8 (25%) of the FTCs. However, intranodular microcalcifications were also observed in 4/27 (15%) of the benign nodules.

The chances of malignancy increased with number of sonographic criteria present. In this study, all except 2/15 (13%) of the malignant lesions had more than one USG
criterion of malignancy. The 2 nodules had microcalcifications as the only USG criterion of malignancy and were histologically proven to be PTCs.

Figure 4.6: “A” showing nodule microcalcifications biopsy confirmed PTC and “B” showing lesion taller than it is wider shape in histologically proven follicular carcinoma.

Figure 4.7: “A” and “B” showing intranodular vascular patterns - false negative and false positive findings respectively.

Figure 4.6: “A” is sonogram of 65 year old female demonstrating fine punctate microcalcifications in the thyroid nodule–histologic confirmed papillary thyroid carcinoma. “B” sonogram of a 43 year old female showing a nodule in the right thyroid lobe that is taller than wider shape–histologic confirmed follicular carcinoma.

Figure 4.7: “A” is a sonogram of a 43 year female showing exclusively perilesional (benign) vascular pattern (type 2) – histology revealed follicular carcinoma. “B” a sonogram of 36 year old female showing marked intralional (malignant) vascularity pattern (type 3) – histology revealed follicular adenoma.
4.4.5 Comparison of $^{99m}$Tc-MIBI and USG:

Tables 4.4 and 4.5 summarise the performance of the two techniques for both benign and malignant nodules controlling for histology.

**Benign nodules:** correct diagnosis of benign lesions were made in 19/27 (70.4%) and 21/27 (77.8%) on $^{99m}$Tc-MIBI scan and USG respectively. This explains that the number of correctly diagnosed benign nodules on $^{99m}$Tc-MIBI and USG (70.4% vs. 77.8%) is not statistically significant ($p > 0.05$).

False positive results were observed in 8/27 (29.6%) and 6/27 (22.2%) for $^{99m}$Tc-MIBI and USG respectively (Table 4.4).

**Table 4.4:** Correlation of $^{99m}$Tc-MIBI and USG in benign lesions (controlled against histology), i.e. histological diagnosis determined the lesions to be benign.

<table>
<thead>
<tr>
<th>MIBI Scan Findings</th>
<th>Number (%) according to USG findings</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Benign</td>
<td>18(66.7)</td>
<td>1(3.7)</td>
</tr>
<tr>
<td>Malignant</td>
<td>3(11.1)</td>
<td>5(18.5)</td>
</tr>
<tr>
<td>Total</td>
<td>21(77.8)</td>
<td>6(22.2)</td>
</tr>
</tbody>
</table>

Note: All findings are not statistically significant ($p$ value >0.05, using McNemar’s Test)

**Malignant nodules:** correct diagnosis was made in 15/15 (100%) and 13/15 (86.7%) on $^{99m}$Tc-MIBI and USG respectively. The number of correctly diagnosed malignant nodules on $^{99m}$Tc-MIBI and USG (100% vs. 86.7%) is again not statistically significant ($p > 0.05$).
There was no false negative result for $^{99m}$Tc-MIBI; there were 2/15(13.3%) false negative for USG (Table 4.5).

These findings tell us that there are no significant differences between $^{99m}$Tc-MIBI and USG performance for both benign and malignant lesions.

**Table 4.5**: Correlation of $^{99m}$Tc-MIBI and USG in malignant lesions (controlled against for histology), i.e. histological diagnosis determined the lesion to be malignant.

<table>
<thead>
<tr>
<th>MIBI Scan Findings</th>
<th>Number (%) according to USG findings</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Benign</td>
<td>0(00.0)</td>
<td>0(00.0)</td>
</tr>
<tr>
<td>Malignant</td>
<td>2(13.3)</td>
<td>13(86.7)</td>
</tr>
<tr>
<td>Total</td>
<td>2(13.3)</td>
<td>13(86.7)</td>
</tr>
</tbody>
</table>

Note -- All findings are not statistically significant (p value >0.05, using McNemar’s Test)

4.4.6 Correlation of a combined ($^{99m}$Tc-MIBI +USG) with histopathologic findings compared with $^{99m}$Tc-MIBI and USG stand-alone.

Tables 4.6 summarises the correlation of histopathological findings with combined ($^{99m}$Tc-MIBI+USG).

When $^{99m}$Tc-MIBI and USG where considered in combination for differentiation benign from malignant STN, 18/27(66.7%) of the histologically proven benign nodules were correctly diagnosed.
**Table 4.6:** Correlation of combined (MIBI + USG) with histopathological diagnosis

<table>
<thead>
<tr>
<th>Combined(MIBI+USG)</th>
<th>Number (%) according to histologic diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Benign</td>
<td>18(66.7)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Malignant</td>
<td>5(18.5)</td>
<td>13(86.7)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>4(14.8)</td>
<td>2(13.3)</td>
</tr>
<tr>
<td>Total</td>
<td>27(100)</td>
<td>15(100)</td>
</tr>
</tbody>
</table>

Note: All findings are not statistically significant (p value >0.05, using McNemar's Test)

The combination achieved a correct diagnosis of 13/15(86.7%) of malignant nodules. However, there were 5/27(18.5%) false positive with no false negative results.

We added a third category as uncertain if the two modalities were not in agreement with each other. Hence 2/15(13.3%) and 4/27(14.8%) respectively of the malignant and benign nodules were classified as uncertain.

The combination modality resulted in increase in specificity (from 70% to 81%) and PPV (from 65 % to 72%) compared to $^{99m}$Tc-MIBI stand-alone; and shows improvement in sensitivity (from 87% to 100%) and NPV (from 91% to 100%) compare to USG stand-alone (see also Table 4.7).

### 4.4.7 Comparison of diagnostic performance these techniques:

The diagnostic performance of $^{99m}$TcO$_4$, $^{99m}$Tc-MIBI, USG, Combined ($^{99m}$Tc-MIBI + USG) and FNA in detecting malignant thyroid nodules – sensitivities, specificities, PPV, NPV and accuracy for all nodules were determined (from the 2 x 2 contingency Tables) using
the following formulae: sensitivity(%) = TP/(TP+FN) x 100; specificity(%) = TN/(FP+TN) x 100; PPV(%) = TP/(TP+FP) x 100; NPV(%) = TN/(FN+TN) x 100; and Accuracy(%) = (TP+TN)/N x 100; where TP, FN,TN and FP are the numbers of true positive, false negative, true negative and false positive cases respectively. N is a statistical denotation for the total number of study population.

The result is presented in Table 4.7.

**Table 4.7:** Diagnostic reliability of $^{99m}$Tc-MIBI, USG, combined (MIBI+USG) and FNA compared against final histologic diagnosis for all tumours.

<table>
<thead>
<tr>
<th></th>
<th>$^{99m}$Tc-MIBI</th>
<th>USG</th>
<th>MIBI+USG</th>
<th>FNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>87</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>70</td>
<td>78</td>
<td>81</td>
<td>100</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>65</td>
<td>68</td>
<td>72</td>
<td>100</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>100</td>
<td>91</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>81</td>
<td>81</td>
<td>81</td>
<td>64</td>
</tr>
</tbody>
</table>

FNA, fine needle aspiration; NPV, negative predictive value; PPV, positive predictive value; $^{99m}$Tc-MIBI, technetium-99m methoxyisobutylisonitrile; USG, ultrasonography.
CHAPTER 5
DISCUSSION

This chapter discusses the research results and relating it with the existing literature. It is presented in the following order: introduction, patients’ demographic information, nodules & histopathologic findings, and correlation / comparison of the various diagnostic modalities with histology.

The summary of the research key findings, conclusion, study limitation and recommendations are also presented here.

5.1 INTRODUCTION

The majority of hypofunctioning STNs are benign with approximate risk for malignancy between 5 to 15\%\textsuperscript{9,10} Identification of this small group of patients is crucial for appropriate and timely treatment - this is prominently true for patients with DTCs since they have excellent prognosis when such treatment is offered on time\textsuperscript{4}.

FNA cytology is sufficient in most cases but it has very low detection rate of FTC\textsuperscript{10,40}. This has also been shown to be very low in our study (12.5\%). This is a serious diagnostic dilemma in our loco-regional population given our epidemiologic scenario of prevalent FTC\textsuperscript{6,7}. The low FNA detection rate of FTC warrants complementary imaging modalities to improve accuracy of thyroid nodule diagnosis.
We feel these imaging modalities are nothing else more than $^{99m}$Tc-MIBI scintigraphy and USG.

5.2 PATIENTS’ DEMOGRAPHIC INFORMATION

A total of 42 patients (39 females and 3 males) between the ages of 19 to 73 years were diagnosed as having clinical STN. There were 39 females and 3 males in this study. Mezosi et al\textsuperscript{10} cohort of 52 patients (49 females and 3 males) between the ages of 16 to 83 years, findings that are similar to our study. Interestingly, 2 of the 3 male patients in our series had malignant thyroid nodules. These findings support the evidence that thyroid lesions are commoner in females,\textsuperscript{35} and male gender is an important risk factor for thyroid cancer.\textsuperscript{25,35,40}

5.3 NODULES AND FINAL HISTOPATHOLOGIC FINDINGS

More than 50% of these nodules were found on the right side. This supports the existing findings in medical literature that the right lobe of the thyroid gland is larger than the left, is more affected by nodules and tends to enlarge more in diffuse goitres.\textsuperscript{81}

In this series, 15/42(35.7\%) patients with hypofunctioning thyroid nodules had final histopathologic diagnosis of malignancy, which is a rather high incidence of cancer than the mostly quoted values.\textsuperscript{3,9,12,65} Hurtado-López et al\textsuperscript{8} however found similar incidence (38.46\%). Although this study was not designed as an epidemiological study, the high frequency of malignancy in our patient population could be explained by the fact that our institution serves as referral centre for other first- and second- level hospitals where
clinicians may have thought that the referred patients could have a malignant thyroid lesion. Secondly most of our patients that underwent surgery had suspicious lesions based on preoperative investigation. Patients whose nodules had no suspicion of malignancy either refused to undergo surgery or even surgeons were reluctant to perform surgery on them - as a result these vast majority patients with benign nodules had to be excluded from the study.

It is also documented that FTC is more common in our institution / region of iodine deficiency.\textsuperscript{6,7,9} This study showed only slight predominance of FTC, 8/15(53.3\%) over PTC, 7/15(46.7\%) – it is unclear if this trend reflects a changing life style of population or as a result of improvement in dietary iodine supplementation. Further epidemiologic studies are essential to validate these findings.

### 5.4 CORRELATION OF THE VARIOUS DIAGNOSTIC MODALITIES WITH HISTOLOGY

The diagnostic modalities here are referring to FNA cytology, \textsuperscript{99m}TcO4\(^{-}\) scintigraphy, \textsuperscript{99m}Tc-MIBI scintigraphy, USG. Also discussed is comparison of USG and MIBI against histopathologic findings, comparison of combined (MIBI + USG) versus either MIBI or USG stand-alone.

#### 5.4.1 FNA cytology

The majority of thyroid nodules can be correctly diagnosed by FNA cytology with accuracy close to 98\.\textsuperscript{60} However, this is not always the case for FTC because
diagnosis relies on whether or not there is vascular or capsular invasion to differentiate between FTC and follicular adenoma. This has resulted in low FNA accuracy rate of about 40% for follicular neoplasia.\(^{40}\)

Our local data showed that FNA cytology diagnosed only 1/8 (12.5%) of FTC and 4/7 (57%) of PTC. This low detection rate of FTC is worrisome especially in our loco-regional population where FTC is the most common primary thyroid malignancy.\(^{6,7}\) This tells us that pre-operative FNA cytology is insufficient, unlike in places where PTC is more prevalent.

Our findings are somewhat similar to the findings of Mezosi et al\(^{10}\) in which FNA cytology was unable to distinguish between FTC and follicular adenoma as none of the 3 FTCs in their series could be diagnosed pre-operatively by FNA cytology. However, correct cytologic diagnoses of 7/9 (78%) of the PTCs was observed in their study.

This inability of cytology to discriminate between carcinoma and adenoma of follicular neoplasia justifies the need for other complimentary imaging modalities such as \(^{99m}\)Tc-MIBI scintigraphy and USG pre-operatively.

5.4.2 \(^{99m}\)TcO\(_4\) scintigraphy

The conventional scintigraphic method of thyroid examination used was \(^{99m}\)TcO\(_4\) scintigraphy. This was not part of the study however, \(^{99m}\)TcO\(_4\) scintigraphy was included in this study to determine the functionality of the nodules and determine which patient would undergo further evaluation with \(^{99m}\)Tc-MIBI scintigraphy. Moreover, dual phase
\(^{99m}\text{TcO}_4^+\) thyroid scintigraphy forms part of the routine scintigraphic evaluation of patients presenting with thyroid diseases in our centre.

Patients that had cold or hypofunctioning thyroid nodule were generally considered in this study. A hypofunctioning thyroid nodule has 5 to 15\%\(^8,^{10}\) risk of malignancy.

5.4.3 \(^{99m}\text{Tc-MIBI scintigraphy}\)

The radiopharmaceutical \(^{99m}\text{Tc-MIBI}\) was introduced in the 1989\(^{10}\) as a myocardial perfusion agent. The tracer was subsequently shown to accumulate in a variety of tumours, including thyroid malignancy.\(^9,^{10,78}\) A property that makes MIBI a potential tumour seeking agent is that it is a lipophilic cation molecule that has high affinity for mitochondria. Accumulation in mitochondria-rich cells such as malignant neoplasia is well established fact and this is driven by mitochondrial negative transmembrane potential.\(^{10}\) Thus MIBI uptake and retention is more common in malignancies than it is in benign conditions.

However there is published evidence suggesting that MIBI is not specific for thyroid cancer, but also accumulates in benign conditions such as adenoma.\(^9,^{78}\) In this study all of the 4/4 (2 follicular and 2 Hürthle cell) adenomas had score 3 MIBI retention similar to what is seen in malignancy. The MIBI retention in benign adenomas is related to high level of mitochondria-rich eosinophilic thyroid cells that are present in these tumours especially the Hürthle cells.\(^82\) Unlike other adenomas, Hürthle cell adenomas show persistent tracer uptake, making them close rival of malignancy.\(^{10}\) Despite accumulation in adenomas, the intense MIBI uptake and retention considerably increases the
probability of differentiated and medullary thyroid cancers, while low or no MIBI uptake drastically reduces the likelihood of (or almost reliably excludes) malignancy.\textsuperscript{9,10,11}

Although this study shows that benign adenomas demonstrated MIBI uptake and retention in a similar fashion as in malignancy, however the interesting finding is that all the 15/15 (100\%) patients with histologically proven carcinomas had positive \textsuperscript{99m}Tc-MIBI scintigraphy. This finding is supported by the previous work of Sathekge et al\textsuperscript{9} that was conducted at our institution. In their study \textsuperscript{99m}Tc-MIBI scintigraphy was positive in 21/23 (91.3\%) of patients with carcinomas, and the 2/23 (8.7\%) malignant lesions that were \textsuperscript{99m}Tc-MIBI scintigraphy negative showed intense uptake on the early 20-minutes images (score 2). If they had considered Mezosi et al\textsuperscript{10} classification of intense early \textsuperscript{99m}Tc-MIBI scan as positive, all of the cancers would have been \textsuperscript{99m}Tc-MIBI scan positive like in our case.

The fascinating findings with \textsuperscript{99m}Tc-MIBI scintigraphy in the evaluation of thyroid nodules are its high NPV and high sensitivity. In this study we found NPV and sensitivity of 100\%. These findings are in conformity with the findings of previously published data in which both sensitivity and NPV were found to be 100\%.\textsuperscript{8,10,69,78,83} High sensitivity (82\% to 96\%) and high NPV (95\% to 97\%) were also reported.\textsuperscript{9,71,74} The high NPV of \textsuperscript{99m}Tc-MIBI entails that negative MIBI scan virtually exclude malignancy in the thyroid nodule and this could obviate the need for unnecessary surgical procedures.

The major limitations of \textsuperscript{99m}Tc-MIBI is the reported wide range of specificity and PPV between 9\% to 88\% and 17\% to 66\% respectively.\textsuperscript{8,10,69,71,74,78,83}
We found specificity of 70% and PPV of 65%. The findings of this study correlate well with a study conducted at our institution by Sathekge et al.\textsuperscript{9} In their study the specificity and PPV were respectively 66% and 77%. Our findings also closely correlate with 61% specificity and 62% PPV obtained by Hurtado-López et al.\textsuperscript{8} The reason for the (disappointing) wide range of specificity and PPV is due to retention of $^{99m}$Tc-MIBI in benign condition such as adenoma. False positive results were seen in 8/27(29.6%) of benign nodules in our series. This is somewhat closer to the false positive findings of 11/48(23%) in Sathekge et al.\textsuperscript{9} study.

Table 5.1 compares the previous $^{99m}$Tc-MIBI studies with our local data.

**Table 5.1** Summary of previous studies showing the diagnostic performance of $^{99m}$Tc-MIBI scintigraphy compared with the current study.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>No. of patients</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>2014</td>
<td>42</td>
<td>100</td>
<td>70</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>Hurtado-Lopez et al.\textsuperscript{8}</td>
<td>2004</td>
<td>130</td>
<td>100</td>
<td>61</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>Sathekge et al.\textsuperscript{9}</td>
<td>2001</td>
<td>71</td>
<td>91</td>
<td>77</td>
<td>66</td>
<td>95</td>
</tr>
<tr>
<td>Mezosi et al.\textsuperscript{10}</td>
<td>1999</td>
<td>52</td>
<td>100</td>
<td>11</td>
<td>28</td>
<td>100</td>
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<tr>
<td>Wale et al.\textsuperscript{69}</td>
<td>2013\textsuperscript{9}</td>
<td>108</td>
<td>100</td>
<td>30</td>
<td>21</td>
<td>100</td>
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<tr>
<td>Wale et al.\textsuperscript{69}</td>
<td>2013\textsuperscript{9}</td>
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<td>96</td>
<td>46</td>
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<td>Theissen et al.\textsuperscript{71}</td>
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<td>154</td>
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<td>Leidig-Bruckner et al.\textsuperscript{74}</td>
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<td>Demirel et al.\textsuperscript{78}</td>
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Wale et al.\textsuperscript{69} (2013\textsuperscript{9})—retrospective review of local data; Wale et al.\textsuperscript{69} (2013\textsuperscript{9})—meta-analysis of the published literature.
5.4.4 Ultrasonography

USG is the imaging modality of choice for evaluation of thyroid abnormalities because of its safety, non-invasiveness, lack of radiation exposure, and easy accessibility. It is the most satisfactory morphological imaging modality that can demonstrate thyroid gland anatomy and often reveals nodules that are clinically impalpable. Sonography can determine whether a nodule is solid or cystic; precisely define the size, site and number of thyroid nodules.

All patients in this study had STN based on clinical examination and all nodules except one were solid on USG. Sonography revealed additional nodule(s) in 16/42 (38%) of cases. This is within the range of reported 20% to 48% of patients with single palpable thyroid nodule have additional nodule(s) on USG.\(^{37}\) Interestingly in our case, 4/16 (25%) of these patients with additional nodule(s) on USG had cancers. Kaur et al.\(^{65}\) shared similar findings in which USG revealed multiple nodules in 16/50 (32%) of patients with STN on palpation. These findings support the idea that discrepancy exists between the true prevalence of thyroid nodules and that obtained by physical examination.\(^{36,37}\)

For this study, we focused on evaluating patients with presumably palpable single thyroid nodule. This is because if we were to exclude all patients with additional nodule(s) on USG, a significant number of patients with thyroid cancers would have been missed. Thus for the purpose of this study we restricted our definition of STN to be a single palpable thyroid nodule regardless of whether or not an additional nodule was present on sonographic evaluation.
For patients that had additional nodules, USG characteristics were evaluated on the dominant nodule corresponding to the site of clinically palpable nodule and/or suspected malignant nodule. We also made sure that the same nodule was assessed with scintigraphic evaluation as well if USG was performed first vice versa.

A thyroid nodule was regarded as malignant if it had one or more of these sonographic criteria including microcalcifications, intranodular vascularity type 3, irregular / microlobulated margin, marked hypoechoogenicity, nodule that is taller than wider shape, and incomplete halo sign as reported in various literatures.\(^2,12,13,23,65,67,75-78\) Although our study was not aimed at appraising individual sonographic criterion, the most frequently encountered criterion in malignant thyroid nodules was intranodular microcalcifications. This was single most important criterion to predict PTC and was found in 86% of our patients with PTC. However, this finding was also present in 25% of our patients with FTC. Similar findings were reported in other studies.\(^75,76,84\)

There is growing evidence to suggest that USG can be helpful for preoperative evaluation of the thyroid nodule and can be used to pre-select patient for FNA cytology, thereby reducing the rate of unnecessary FNA cytologies.\(^75,76,84\) This means that USG has far gone beyond just characterising the nodule as either solid or cystic as initially thought by many surgeons / physicians.

The specificity of USG in differentiating benign and malignant thyroid nodules in our study is 77.8%. This finding correlates well with studies of Kaur \textit{et al.},\(^65\) and Lingam \textit{et al.}\(^84\) Higher specificity of 90.6% was recorded by Kioke \textit{et al.}\(^77\) We found sensitivity of
86.7%, this is well within the reported values of 71.4% to 100% obtained in other studies.\textsuperscript{65,75,77,84} The NPV of USG in our study was a bit higher (91.3%) compared to other parameters; this is comparable to 95.9% NPV obtained by Kim et al.\textsuperscript{75} In Lingam \textit{et al.}\textsuperscript{84} study the NPV was also found to be 100%.

These findings suggest that sonography can be helpful in differentiation of benign from malignant lesions. Thus USG in combination with $^{99m}$Tc-MIBI scintigraphy and FNA cytology will lead to optimal results.

Table 5.2 compares the previous USG studies with our local data.

\textbf{Table 5.2} Summary of previous studies showing the diagnostic performance of USG compared with the current study.

\begin{table}[h]
\centering
\begin{tabular}{llcccc}
\hline
Authors & Year & No. of patients & Sensitivity(\%) & Specificity(\%) & PPV(\%) & NPV(\%) \\
\hline
This study & 2014 & 42 & 86.7 & 77.8 & 68.4 & 91.3 \\
Kaur et al.\textsuperscript{65} & 2002 & 50 & 71.4 & 77.7 & - & - \\
Kim et al.\textsuperscript{75} & 2002 & 132 & 93.8 & 66.0 & 56.1 & 95.9 \\
Kioke et al.\textsuperscript{77} & 2001 & 309 & 81.8 & 90.6 & - & - \\
Demirel et al.\textsuperscript{78} & 2003\textsuperscript{f} & 43 & 100 & 76.0 & 53.0 & 100 \\
Erdem et al.\textsuperscript{79} & 1997\textsuperscript{g} & 26 & 100 & 85.0 & 100 & 62.0 \\
Lingam et al.\textsuperscript{84} & 2013 & 215 & 100 & 76.0 & 33.0 & 100 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{Demirel et al.\textsuperscript{78} (2003) and Erdem et al.\textsuperscript{79} (2013) — only Doppler USG criteria as predictor malignant nodule were considered.}
5.4.5 Comparison of $^{99m}$Tc-MIBI and USG / combined ($^{99m}$Tc-MIBI and USG) with $^{99m}$Tc-MIBI scintigraphy and USG stand-alone.

In this study, when $^{99m}$Tc-MIBI scintigraphy was compared with USG, $^{99m}$Tc-MIBI scan was found to be more sensitive and with higher NPV. Both the sensitivity and NPV for $^{99m}$Tc-MIBI scintigraphy were 100%; and sensitivity and NPV for USG were 86.7% and 91.3% respectively. However, these findings were not statistically significant ($p >0.05$). To the best of our understanding and with extensive search of literature, we have not come across a single study that compared $^{99m}$Tc-MIBI scintigraphy and USG (with both grey scale and Doppler USG features).

The closest comparison of MIBI and US is the study by Demirel et al\textsuperscript{78} where they compared radionuclide thyroid angiography, $^{99m}$Tc-MIBI scintigraphy and PDUS in evaluation of solitary cold thyroid nodules. In this, the Authors considered only the power Doppler features. They found that both the sensitivity and NPV for PDUS to be 100% while it was 78% and 93% for $^{99m}$Tc-MIBI scintigraphy. Similarly Cox et al\textsuperscript{80} evaluated STN using $^{99m}$TcO$_4^-$ scintigraphy and USG. In their study they did not include $^{99m}$Tc-MIBI study.

The combination of $^{99m}$Tc-MIBI scintigraphy and USG were compared with $^{99m}$Tc-MIBI scintigraphy stand-alone or USG stand-alone. The combined ($^{99m}$Tc-MIBI + USG) modalities showed slight rise in specificity (80.77% versus 70%) and PPV (72.2% versus 65%) compared to $^{99m}$Tc-MIBI stand-alone; and there was much increase in
sensitivity (100% versus 86.7%) and NPV (100% versus 91.3%) compared to USG stand-alone (Table 4.7).

As summarised in Table 4.7, the $^{99m}$Tc-MIBI scintigraphy, USG and combined ($^{99m}$Tc-MIBI + USG) compared with FNA cytology, FNA had better specificity (100%) compared to 70% for MIBI; 77.8% for USG; and 80.77% for combined and better PPV (100%) compared to 65% for MIBI; 68.4% for USG; and 72.2% for combined. The sensitivity of FNA was however, lower (83.3%) compared to 100% for MIBI; 86.7% for USG; and 100% for combined. FNA had lower accuracy rate of 64% compared to all the three methods which had accuracy of 81% each.

### 5.5 SUMMARY OF THE KEY FINDINGS

The findings of this study are summarised as follow:

- Forty-two (42) patients with clinical STN were evaluated. Majority of the patients were in the age group of 31 to 50 years.
- There were 39 females meaning that thyroid diseases / nodules are more prevalent in women as reported in literature.
- Two of the only 3 male patients in our cohort had cancers supporting the existing literature that male gender is an important risk factor for thyroid cancer.
- Most nodules (67%) in our series occurred in the right thyroid lobe. There is evidence in literature to suggest that the right lobe of the thyroid is larger and most likely to be affected in thyroid disease / nodules than the left lobe.
There were 15/42 (35.7%) thyroid carcinomas; 8/15 (53%) FTC and 7/15 (46.7%) PTC (including 4 follicular variant and 3 classical papillary).

FNA cytology was diagnostic in 5/15 (33%) of all cancers; in 1/8 (12.5%) of the FTC and 4/7 (57%) of PTC. This supports the findings in literature that FNA detection capability of FTC is very low.

99mTc-MIBI scintigraphy was positive in all, 15/15 (100%) of the malignant nodules but most importantly it had high NPV (100%) and sensitivity (100%). However, the PPV was lower (65%) and specificity was 70%.

USG was positive in 13/15 (86.7%) of the malignant nodules with reasonable NPV (91%) and sensitivity (87%). The specificity and PPV were respectively 78% and 68%.

There was no statistically significant difference between 99mTc-MIBI and USG performance (p > 0.05).

Combination modalities (MIBI + USG) resulted in slight increase in specificity and PPV respectively 81% and 72%.

5.6 CONCLUSION

The combined performance of 99mTc-MIBI scintigraphy and USG is complementary to FNA cytology to improve pre-operative diagnostic accuracy. This is significantly important in areas where FTC predominates, largely developing population or in a setting of inconclusive cytology.
The low 12.5% FNA cytology detection of FTC implies its low sensitivity overall and thus supports the need for extended evaluation using $^{99m}$Tc-MIBI and USG. The high NPV of MIBI scintigraphy together with USG can usefully exclude malignancy when FNA is non-diagnostic and may provide further reassurance when FNA suggests benignity.

5.7 LIMITATIONS OF THE STUDY

The relatively small sample size is a limitation of the study. The study cohort was selected on the basis of the decision to proceed to thyroid surgery and did not include all consecutive patients with hypofunctioning nodules on $^{99m}$TcO$_4$ thyroid scintigraphy. This was largely due to some logistic problem with the operating theatre space at DGMAH.

This low cohort is because most patients operated are the ones that had very high suspicion for malignancy; and hence leaving the majority of patients with benign nodule. Thus, accounting for the higher incidence of malignancy in this study than is found in standard population with hypofunctioning thyroid nodules: 37.5 % (15/42).

There is limited literature on the evaluation of thyroid nodules using combined $^{99m}$Tc-MIBI scintigraphy and USG to validate our findings.

5.8 RECOMMENDATIONS

- Further data is essential to validate the combined role of $^{99m}$Tc-MIBI scintigraphy with USG, perhaps with larger sample size.
We recommend that $^{99m}$Tc-MIBI scintigraphy be included as part of the routine diagnostic work up of STN particularly if there is clinical suspicion of malignancy.
REFERENCES


42. Gharib H, Papini E. Thyroid Nodules: Clinical Importance, Assessment, and Treatment. Endocrinol Metab Clin N Am. 2007; 36: 707-735

44. Guidelines for Surgery: Tumors of Follicular Cell Origin


69. Wale A, Miles KA, Young B et al. Combined (99m)Tc-methoxyisobutylisonitrile scintigraphy and fine-needle aspiration cytology offers an accurate and


APPENDICES

Appendix 1: Statistician endorsement

STATISTICAL ANALYSES

The Chairperson,
Medunsa Research Ethics Committee (MREC),
Box 163
UNIVERSITY OF LIMPOPO
Medunsa Campus

Dear Sir

STATISTICAL ANALYSES
I have studied the research protocol of Dr \textbf{A. Farate} titled: \textit{Evaluation of Solitary Thyroid Nodules with Technetium-99m Methoxy-isobutyl-isonitrile and Ultrasonography} and I agree to assist with the statistical analyses.

Yours sincerely,

Signature: Statistician

Name in block letters
28 August 2012
Date
Appendix 2: MREC protocol approval certificate

UNIVERSITY OF LIMPOPO
Medunsa Campus

MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

MEETING: 02/2013
PROJECT NUMBER: MREC/M/2013: PG
PROJECT:
Title: Evaluation of solitary thyroid nodule with Technetium-99m Methoxyisobutyl-isonitrile and Ultrasonography: The Dr George Mukhari Hospital experience

Researcher: Dr A Farate
Supervisor: Dr AA Guttta
Co-supervisor: Prof T Mdaka
Hospital Superintendent: Dr NME Sithole (Dr George Mukhari Hospital)
Other involved HOD: Prof ME Kisansa (Radiology)
Department: Radiology, Nuclear Medicine, Diagnostic Radiography & Medical Bio-Physics
School: Medicine
Degree: MMed Nuclear Medicine

DECISION OF THE COMMITTEE:
MREC approved the project.

DATE: 07 March 2013

PROF PGD RAUTENBACH
DEPUTY CHAIRPERSON MREC

The Medunsa Research Ethics Committee (MREC) for Health Research is registered with the US Department of Health and Human Services as an International Organization (IORG0004319), as an Institutional Review Board (IRB00000532), and functions under a Federal Wide Assurance (FWA00009419)
Expiry date: 11 October 2016

Note:

i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.

ii) The budget for the research will be considered separately from the protocol. PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.
Appendix 3: Hospital clinical management permission

To: Dr. Abubakar Farate
Department of Nuclear Medicine
P.O. Box 83
University of Limpopo
MEDUNSA
0208

Date: 06 September 2012

Permit to Conduct Research

The Dr. George Mukhari Hospital hereby grants you permission to conduct research on "Evaluation of Solitary Thyroid Nodule with Technetium-99m Methoxy-Isobutyl-Isocitrile and Ultrasonography".

This permission is granted subject to the following conditions:

☐ That you obtain Ethical Clearance from the Human Research Ethics Committee of the relevant University
☐ That the Hospital incurs no cost in the course of your research
☐ That access to the staff and patients at the Dr George Mukhari Hospital will not interrupt the daily provision of services.
☐ That prior to conducting the research, you will liaise with the supervisors of the relevant sections to introduce yourself (with this letter) and to make arrangements with them in a manner that is convenient to the sections.

Yours sincerely

DR. P. SHEMBE
DIRECTOR: CLINICAL SERVICES
DEPARTMENT OF DIAGNOSTIC RADIOLOGY & IMAGING
P.O. Box 63, Medunsa 0204 South Africa
Tel: 27 12 529 3872
Fax: 27 12 560 0484
Enquiries: Mrs Esther Barnard
Email: radiology@medunsa.ac.za
Ref. 05/05/2012

Dr. Abubakar Farate
Department of Nuclear Medicine
2nd Floor
MEDUNSA
0204

Dear Dr. Farate,

Permission has been granted for you to collaborate with doctors in the Department of Diagnostic Radiology and Imaging on your project.

Kind regards

Prof. M E KISANSA
HEAD OF DEPARTMENT

11 May, 2012
Appendix 5: Data sheet

1. Patient's demographic data:

Study No._______ Age_______ Gender_______ Res (NMR). No__________

Contact No._________ Ward/Clinic________

2. Thyroid exam..........................................................................................................................
..............................................................................................................................................
..............................................................................................................................................

3. Scintigraphic assessment criteria:

i. $^{99m}$TcO$_4^-$ scintigraphy: [mark “X” or tick].

Flow / uptake mismatch_________ Flow / uptake match__________
Perfusion / uptake mismatch was considered malignant whereas flow / uptake matched pattern was considered benign.

ii. $^{99m}$Tc-MIBI scintigraphy *4-point score: [mark “X” or tick].

Score 0_______ Score 1_______ Score 2_________ Score3_______

- **Score 0**: No significant uptake
- **Score 1**: Uptake increased compared with the background activity but lower than normal thyroid
- **Score 2**: Uptake equal to the normal thyroid
- **Score 3**: Uptake superior to normal thyroid with retention on delayed 2-hour image.

Scores 0 to 2 was considered benign whereas score 3 was considered positive.

4. **USG assessment criteria:**

Sonographic characteristics of the nodule:

Size________   Echogenicity________   Margin_________
Calcification________ Shape_________ Vascularity type/RI__________ other findings_________ Remark_______
USG findings in favour of malignancy:

- Marked hypoechogenicity solid nodule: when nodule is markedly hypoechoic, with darker appearance compared to adjacent strap muscles.
- Irregular or microlobulated margin and incomplete halo sign.
- Microcalcifications: fine punctate / snowstorm calcification due to calcified psammoma bodies.
- Shape taller than wider: nodule with anteroposterior (AP) diameter greater than the transverse diameter.
- Type 3 intranodular vascularity: nodule showing central flow with multiple vascular poles chaotically arranged with or without significant perinodular vessels.

A nodule showing one or more of the features above was considered malignant whereas if none of the features was present the nodule was considered benign.

USG findings in favour benign:

- Hyperechoic nodule
- Nodule that has smooth margin
- Large coarse / peripheral / egg-shell calcifications in the nodule
- The nodule with shape that is wider than it is taller shape configuration(i.e. the transverse diameter greater than AP diameter)
• A nodule with absent flow or exclusively perinodular vascularity (types 1 and 2 respectively)

The above features were considered benign in the absence of malignant features listed above.

5. **Histopathological assessment:**

**FNA findings:**

- Benign____ Follicular neoplasm_____ Malignant______ Inconclusive____
  Others____

**Final histopathologic findings:**

- Benign____ Malignant ______ others _____Hist. cell
  type______________
Appendix 6: Patient information and informed consent

PATIENT INFORMATION AND INFORMED CONSENT DOCUMENT

Project number: MREC/M/20/2013: PG

Title of the project: Evaluation of solitary thyroid nodule with technetium-99m methoxyisobutylisonitrile and ultrasonography at Dr. George Mukhari Academic Hospital.

Introduction

You are requested to take part in a study. This document will help you to decide whether you would like to participate. Before you agree to take part in this study you should fully understand what it is all about. If you have any questions that are not fully explained in this document, do not hesitate to ask the study doctor. You should not agree to take part unless you are completely happy about all the procedures involved.

What is the purpose of this study?

You have been identified with a growth in front part of your neck (thyroid). Ultrasound (sonar) and thyroid (nuclear) scans form part of routine examinations. This will also require a needle be introduced into the growth to take out small piece of it for cytologic analysis (FNA). The above efforts is to find out if your neck growth is a cancer or not and based on which decision for further treatment will be taken.
How will these examinations performed?

The procedures go as follows: You will have two nuclear scans one week apart, sonar and in the following days or week you will have FNA. For the nuclear scan an intravenous (IV) line will be inserted in your arm. This is similar to a pinprick when having blood drawn. The radiopharmaceutical ($^{99m}$Tc$O_4^-$) is then injected through the IV line after which you will be put into gamma camera (machine) to take pictures of your neck immediately and at 20 minutes after injection. This is repeated the following week but with different radiopharmaceutical called $^{99m}$Tc-MIBI and pictures are taken at 20 minutes and 2 hours after injection. For USG you will be asked to lie on your back with your neck extended and a thin non-harmful semi-liquid jell will be applied on the growth. An ultrasound probe will then be moved gently over the growth and rest of the thyroid - pictures will also be taken.

Is there risk associated with these exams?

A radiopharmaceutical is a very small amount of pharmaceutical that is tagged with a tiny amount of radioactive material. The amount of radiation you will receive from a $^{99m}$Tc$O_4^-$ or $^{99m}$Tc-MIBI study is equivalent to the amount you would receive from other conventional scans that have been used for decades. There is no published evidence to suggest any side effects in humans that are directly related to radiation exposure to these radiopharmaceuticals. Your body is able to quickly eliminate the radioactive materials used. USG is free of ionising radiation and there is no known risk associated with its use.
What is the duration of this research?

We will enroll patients to this study, which will probably be completed in 12 months.

Has the study received ethical approval?

The protocol of this research project was approved by Medunsa Research Ethics Committee of the University of Limpopo. The study has been structured in accordance with MREC and Committee of Helsinki (last updated in 2013), which deal with the recommendations guiding doctors in biomedical research involving human participants. Copies of these documents can be obtained from a study doctor should you wish to review it.

What are your rights as a participant in this study?

Your participation in this trial is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other medical care. The study doctor retains the right to withdraw you from the study if it is considered to be in your best interest.

May any of these research procedures result in discomfort or inconvenience?

Installation of the intravenous line might be uncomfortable and even slightly painful. It might also result in bruise formation and infections. Administration of the radiopharmaceuticals (\(^{99m}\text{TcO}_4^-\) or \(^{99m}\text{Tc-MIBI}\)) in the vein might be uncomfortable and even slightly painful. It might also result in bruise formation and infections. All these
procedures will however be done only by well-experienced health personnel, under hygienic conditions, and in your best interest. USG is generally has less discomfort except for few cases where growth may be painful – gentle and skillful examination is necessary.

**Costs**

You will not be paid to participate in this trial. You will also not be asked to pay anything more than the usual fees required for admission to hospital.

**Confidentiality**

All information obtained during the course of this study is strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a patient in this study. You will be informed of any finding of importance to your health or continued participation in this research.

Any concerns or questions can be directed to:

Dr A Farate (Researcher)
Department of Nuclear Medicine
University of Limpopo
MEDUNSA Campus
Tel. (work): 0125215753
Mobile: 0730931654
Email: abu.farati@gmail.com
INFORMED CONSENT

I have read the information on the proposed project and was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurised to participate in any way. I know that electronic images will be taken of me if need be. I am aware that this material may be used in scientific publications which will be electronically available throughout the world. I consent to this provided that my name is not revealed.

I understand that participation in this project is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I know that this Project has been approved by the Medunsa Campus Research and Ethics (MREC), University of Limpopo (Medunsa Campus) / Dr George Mukhari Hospital. I am fully aware that the results of this project will be used for scientific purposes and may be published. I agree to this, provided my privacy is guaranteed.

I hereby give consent to participate in this study.

Patient / Guardian (signature) ___________________________ Date ________________

Person obtaining informed consent (signature) _____________ Date ________________

Witness (signature) ________________________________ Date ________________
VERBAL PATIENT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned, Dr ……………………………., have read and have explained fully to the
patient, named ……………………………………………and / or his / her relative, the
patient information leaflet, which has indicated the nature and purpose of the study in
which I have asked the patient to participate. The explanation I have given has
mentioned both the possible risks and benefits of the study and the alternative
treatments available for his / her illness. The patient indicated that he / she understand
that he / she will be free to withdraw from the study at any time for any reason and
without giving reasons.

I hereby certify that the patient has agreed to participate in this study.

Patient’s Name ________________________________
(Please print)

Investigator’s Name ________________________________
(Please print)

Investigator’s Signature_________________________ Date________________

Witness’s Name ________________________________
(Please print)

Witness’s Signature_________________________ Date________________