A CORRELATION STUDY OF CYTOPATHOLOGY AND HISTOPATHOLOGY AND THE ASSOCIATED RISKS FOR MALIGNANCY IN THYROID NODULE

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Dissertation Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master Of Medicine (General Surgery)

UNIVERSITI SAINS MALAYSIA

2017

ACKNOWLEDGEMENTS

In the name of Allah SWT, Most Gracious, Most Merciful

Praise to Allah s.w.t., the most gracious and most compassionate, whose blessing have helped me through the entire completion of this research project. I would like to express my utmost gratitude to the following individuals who have helped me during the preparation of this research project report and during the course to pursue Master of Surgery in School of Medical Science, Universiti Sains Malaysia, Kelantan.

- Dr. Maya Mazuwin Yahya, Supervisor and Lecturer, Breast and Endocrine Surgeon, Department of Surgery, USM
- Dr. Imi Sairi Ab. Hadi, Co-supervisor and Consultant Breast and Endocrine Surgeon, Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan
- All lecturers in the Department of Surgery and Department of Community Medicine who have given guidance, knowledge and support for the dissertation and the course
- My beloved wife and children, my beloved parents and in laws, and all Master of Surgery colleagues who were willing to help and give their support and inspiration during this study

Thank you very much.

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IV. LIST OF ABBREVIATIONS AND NOMENCLATURES

FNAC	Fine Needle Aspiration Cytology
ATA	American Thyroid Association
USG	Ultrasonography
FNA	Fine Needle Aspiration
AUS	Atypia of Undetermined Significant
FLUS	Follicular Lesion of Undetermined Significant
TSH	Thyroid Stimulating Hormone
STN	Solitary Thyroid Nodule
MNG	Multinodular Goitre
ROC	Receiver Operation Characteristics
AUC	Area Under Curve
MTC	Medullary Thyroid Cancer
AOR	Adjusted Odd Ratios
HRPZ II	Hospital Raja Perempuan Zainab II
RLN	Recurrent Laryngeal Nerve
OR	Odd Ratio
CI	Confidence Interval
LR	Likelihood Ratio
TFT	Thyroid Function Test
SD	Standard Deviation

AACE	American Association of Clinical Endocrinologist
AME	Associazione Medici Endocrinologi
ETA	European Thyroid Association
FPR	False Positive Rate
FNR	False Negative Rate
PTC	Papillary Thyroid Carcinoma
mPTC	Micropapillary Thyroid Carcinoma

V. ABSTRAK BAHASA MALAYSIA

Pengenalan:

Benjolan tiroid telah direkodkan meningkat di seluruh dunia disebabkan pelbagai faktor. Di dalam menetapkan kaedah perawatannya, 'Fine needle aspiration cytology' (FNAC) dan pemeriksaan histopatoloji harus dipraktikkan untuk menentukan diagnosis tisu yang tepat. Lebih kurang 10-20% keputusan FNAC tiroid jatuh ke dalam kategori Bethesda III & IV dan meningkatkan risiko barah sebanyak 5-30%. Kami berhasrat untuk menentukan tahap kejadian dan petunjuk klinikal terhadap barah bagi keputusan kumpulan FNAC ini.

Objektif:

Objektif utama adalah untuk mencari aras persetujuan di antara sitopatoloji dan histopatoloji di kalangan pesakit pembedahan tiroid dan mengenalpasti petunjuk klinikal di kalangan pesakit dengan keputusan FNAC Bethesda III & IV.

Kaedah:

Demografik data semua pesakit yang menjalani pembedahan tiroid dengan prosedur FNAC sebelum pembedahan di antara bulan Jun 2007 sehingga May 2013 di Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan telah disemak. Keputusan akhir histopatoloji telah dianalisis dan dipertimbangkan. Tahap spesifisiti dan sensitiviti FNAC telah dikira dan direkodkan. Risiko petunjuk barah di kalangan sitoloji meragukan termasuklah umur, jantina, bangsa, status tiroid, jangkamasa, benjolan dengan simtom dan sifat benjolan telah diteliti. Semua data telah dianalisis menggunakan analisis regresi logistik berganda.

Keputusan:

Sejumlah 333 pesakit (purata umur ± sisihan piawai, 44.07 ± 14.3 tahun) telah terlibat dengan nisbah jantina 1:4 (lelaki: perempuan), dan kebanyakan pesakit berbangsa Melayu (94.6%). Purata jangkamasa (sisihan piawai) untuk benjolan tiroid adalah 4.72 (6.007) tahun. Perbandingan di antara sitopatoloji dan hsitopatoloji menepati tahap kekuatan yang sangat baik (Cohen's Kappa analisis, κ= 0.751 (95% CI: 0.671, 0.831). Tahap sensitiviti, spesifisiti, nilai jangkaan positif, nilai jangkaan negatif, nisbah kesalahan positif, nisbah kesalahan negatif dan jumlah ketepatan adalah seperti berikut 89%, 91%, 76%, 96%, 9%, 11% and 90%, secara berturutan. Barah paling ketara adalah barah tiroid papilari dengan penglibatan 60 pesakit (74.1%).

Di kalangan pesakit dengan sitoloji Bethesda III & IV, telah didapati faktor banyak benjolan (58% dan 42%, p = 0.036) meningkatkan risiko barah yang signifikan. Walaubagaimanapun, faktor umur, jantina, bangsa, status tiroid, jangkamasa dan benjolan dengan simtom telah didapati tidak meningkatkan risiko barah.

Kesimpulan:

Benjolan tiroid telah didapati meningkat dan FNAC adalah 'gold standard' prosedur sebelum pembedahan yang amat tepat. Di kalangan pesakit dengan keputusan sitotoji Bethesda III & IV, kami mendapati faktor banyak benjolan meningkatkan risiko barah.

VI. ABSTRACT IN ENGLISH

Introduction:

Thyroid nodule incidence has been reported increasing worldwide due to various factors. In order to determine the management of this entity, Fine needle aspiration cytology (FNAC) and histopathology examination must be performed to correctly identify the tissue diagnosis. Approximately 10-20% of thyroid FNAC diagnoses fell into Bethesda III & IV category and carried 5-30% risk of malignancy. We aim to identify the occurrence and clinical predictors towards malignancy in these groups.

Objective:

The main objective is to study the agreement between cytopathology and histopathology in thyroidectomy patients and to identify the associated risks of malignancy in patients with Bethesda III & IV thyroid nodule.

Methods:

Patients who underwent thyroidectomy with prior FNAC between June 2007 and May 2013 in Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan were retrospectively reviewed demographically. Final histopathology were analyzed and compared. The specificity and sensitivity study of thyroid FNAC were calculated and recorded. The associated risk for malignancy in indeterminate cytology nodule including age, gender, race, thyroid status, duration, symptomatic nodule and nodularity were reviewed.

Results:

A total of 333 patients (mean age ± SD, 44.07 ± 14.3 years) were enrolled with gender ratio of 1:4 (male to female), and majority of patients were Malay ethnic (94.6%). Mean duration of thyroid nodule was 4.72 years (SD = 6.007). The correlation between cytopathology and histopathology has substantial strength of agreement (Cohen's Kappa analysis, κ= 0.751 (95% CI: 0.671, 0.831). The sensitivity, specificity, positive predictive value, negative predictive value, false positive rate, false negative rate and total accuracy of FNAC were 89%, 91%, 76%, 96%, 9%, 11% and 90%, respectively. Commonest malignancy detected was papillary thyroid carcinoma in 60 patients (74.1%).

In patients with cytology Bethesda III & IV, the presence of multiple nodule (58% vs. 42%, p=0.036) was associated with higher malignancy risk. Neither age, gender, race, thyroid status, duration nor symptomatic nodule were associated with malignancy risk.

Conclusion:

Thyroid nodule is predicted higher incidence and FNAC is gold standard preoperative procedure with high accuracy. In patients with Bethesda III & IV cytopathology, we noted the presence of multiple nodules was associated with increased risk for malignancy.

CHAPTER 1:

INTRODUCTION

Thyroid nodule is a common clinical presentation in daily surgical practice. It is also considered as the most common endocrine tumour in comparison with other endocrine pathology (Bryan McIver 2013). According to American Thyroid Association (ATA) thyroid nodule is defined as "discrete lesion within the thyroid gland and radiologically distinct from surrounding thyroid parenchyma" (David S. Cooper, Gerard M. Doherty et al. 2009). It has been historically found commonly by palpation during a routine general physical examination due to patients' concern and awareness. However non-palpable nodule has been increasingly discovered with radiographic studies performed for other medical evaluations which termed incidentally discovered nodules or "incidentalomas" (David S. Cooper, Gerard M. Doherty et al. 2009). Radiographic studies such as carotid duplex ultrasound (USG), computed tomography scan, magnetic resonance imaging studies, or 18-fluorodeoxyglucose positron emission tomography scanning which were done to evaluate other non-thyroid pathology has provide the findings of incidentalomas.

Thyroid nodule whether single or multiple has become more prevalent as reported in many studies. The estimated prevalence by palpation alone ranges from 4% to 7% (Popoveniuc and Jonklaas 2012), and with the current use of high resolution USG techniques, they can detect nodule 20% to 76% of adult population (Steven R. Bomeli, Shane O. LeBeau et al. 2010). In United States, the estimated annual incidence of thyroid nodule is approximately 0.1% per year, 4 times more common in female and the frequency increases with age and low iodine intake (Popoveniuc and Jonklaas 2012). It is also proved in autopsy series that fewer than 5% of all

thyroid nodules, whether it is palpable of incidentally discovered, does harbour malignant cells (Steven G. Silverberg and Romeo A. Vjodone 1966, Chung-Che Charles Wang, Lyssa Friedman et al. 2011, Popoveniuc and Jonklaas 2012, Bryan McIver 2013).

In Malaysia, National Cancer Registry 2006 reported cancer incidence of thyroid malignancy occurred 2.2 per 100,000 and 6.8 per 100,000 for male and female respectively (Zainal Ariffin Omar, Zainudin Mohd. Ali et al. 2006). Another latest review on prevalence of endemic goiter in Malaysia done in 2012, it was reported that high incidence of goiter in seven states: Sabah, Sarawak, Kelantan (author's state of interest), Terengganu, Pahang, Perlis and Kedah (Than 2012). Thyroid cancer incidence also noted to be prevalently increasing with papillary thyroid cancer remains the most common cancer reported. A study on hospital-based incidence of thyroid lesions in detecting carcinoma, thyroid malignancy accounts for 3.5 per 100,000 among 1,486 thyroid specimens sent for cytopathological assessment (Othman, Omar et al. 2009). Another large scale study was done in Kelantan state in year 1993 by Universiti Sains Malaysia, Kelantan, the prevalence of goiter was found overall 36.8% among the whole state (M. Mafauzy, W.B. Wan Mohamad et al. 1993). Since the reported prevalence is high, it is always relevant to project the morbidity that might rised, therefore earlier prevention measures could be undertaken.

The pathogenesis of thyroid nodule has been well described and studied in many previous literatures. It has been postulated to be considered as an amplification of thyroid heterogeneity due to genetic and/or epigenetic mechanisms that is further classified into five types; hyperplastic, neoplastic, colloid, cystic and thyroiditic nodule (Salabe 2001). With the main concern on neoplastic thyroid nodule, it is well known that mutations causing activation of proto-oncogenes to oncogenes are a fundamental step in carcinogenesis. Several activated proto-

oncogenes had been identified and closely relevant to thyroid malignancies; ras oncogene family, gsp, RET, c-MET, TRK, EGF/ EGF-R and p53 (Salabe 2001). This is the most important value in correctly identifying those patients who fall under different types of nodule histologically and prompt further evaluation and surgical intervention.

When a patient presented with thyroid nodule, many differential diagnoses can be made. The most common diagnoses are colloid nodule/ thyroiditis (80%), benign follicular neoplasms ("adenoma" 10-15%), cysts and thyroid carcinoma (5%). Other less common diagnoses as listed below.

Table 1.1 Thyroid nodule - Differential diagnosis

Neoplastic thyroid diseases
Benign
Thyroid (follicular/ Hu¨rthle cell) adenoma
Malignant
Thyroid cancer
Primary (papillary, follicular [including Hurtle cell)
Metastatic
Inflammatory thyroid disease (thyroiditis)
Acute
Subacute
Hashimoto
Riedel disease
Infectious thyroid disease
Abscess
Tuberculosis
Granulomatous disease
Developmental abnormalities
Thyroglossal duct cyst
Teratoma
Other benign thyroid diseases
Colloid (adenomatoid) nodule
Thyroid cyst
Graves' disease

Adapted from (George H. Sakorafas, George Peros et al. 2006)

The primary challenge in managing thyroid nodules is to reliably identify the majority of benign nodules that do not require intervention or surgical removal. There are several clinical implications of thyroid nodule. They may cause thyroid dysfunction and compressive symptoms but the main concern is the need to exclude thyroid malignancy. According to reported studies, the prevalence of malignancy in thyroid nodule evaluated by biopsy ranges from 4.0% to 10% (Chih-En Tseng, Chang-Kuo Wei et al. 2008, Chung-Che Charles Wang, Lyssa Friedman et al. 2011). This high incidence suggests that although it is small in size but it may carry high mortality and morbidity in the future. Concerning that thyroid nodule has the probability to be malignant, tissue biopsy is crucial to determine which nodules need further surgical intervention.

Fine needle aspiration cytology (FNAC) is the most important step in the workup of the thyroid nodule. FNAC is widely available, cost effective, easily repeated and quick to perform procedure in the setting of outpatient department. It can be performed with or without ultrasound guidance, however lower rates of both non-diagnostic and false negative cytology specimen has been reported with the use of ultrasound (Elniel H. Eltyib, A. Awad et al. 2014). FNAC has its own limitation and pitfalls; accuracy is lower in suspicious cytology and in follicular neoplasm. The quality and adequacy of the FNAC specimen can be influenced by sampling errors and by intrinsic factors of nodules, such as small size, hypervascularity, cystic changes, calcification and fibrosis (Leea, Parka et al. 2015). The only malignant pathology that is reliably diagnosed through fine needle aspiration is papillary thyroid carcinoma, because of th characteristic features such as “Orphan Annie” nuclei, nuclear grooves, intranuclear inclusions, and psammoma bodies can be pathognomonic for a diagnosis. Benign and follicular neoplasms and oncocytic (formerly called Hurthle cell) adenomas and carcinomas cannot be distinguished by cytology alone, in view of tissue architecture is required to make a diagnosis of malignancy

through observation of capsular or angiolymphatic invasion in a tissue biopsy sample (Steven R. Bomeli, Shane O. LeBeau et al. 2010). Recent advances in the application of molecular markers to FNAC are changing the principles in diagnosing the suspicious nodule in which it is not widely available.

According to The Bethesda System for Reporting Thyroid Cytopathology, fine needle aspiration (FNA) classification scheme fall into 6 categories; nondiagnostic or unsatisfactory, benign, atypia of undeterminate significance (AUS) or follicular lesion of undetermined significance (FLUS), follicular neoplasm or suspicious for a follicular neoplasm, suspicious for malignancy and malignant. Each category carries risk percentage of malignancy; 1-4% in nondiagnostic, 0-3% in benign, 5-15% in AUS, 15-30% in follicular neoplasm, 60-75% in suspicious for malignancy and 97-99% in malignant (Edmund S. Cibas and Syed Z. Ali 2009) . However these value ranges accordingly as many studies looking at false negative FNAC and has been reported as high as 45.8% (Joanna L. Morgan, Jonathan W. Serpell et al. 2003). In view of these discrepancy and pitfalls, it raised a concern about looking back into local data in correlating cytopathology and histopathology results to identify specifically non-neoplastic cytology that turned up to be malignant.

Multiple risk factors had influenced a benign thyroid nodule to become malignant. Many literatures had studied on variety of objective and subjective risk factors that may give an impression towards malignancy changes. According to ATA guideline, risks factors such as age younger than 20 or older than 70 years, male gender, large nodule size, and presence of lymphadenopathy carries higher risk of malignancy (David S. Cooper, Gerard M. Doherty et al. 2009). A recent prospective study conclude that higher serum thyroid stimulating hormone (TSH) levels at presentation even within normal range is an independent predictor of the

presence of thyroid malignancy (K. Boelaert, J. Horacek et al. 2006). The main historical factors include history of childhood head and neck irradiation, total body irradiation or bone marrow transplantation, family history of thyroid carcinoma or thyroid cancer syndrome (e.g., Cowden's syndrome, familial polyposis, Carney complex, multiple endocrine neoplasia 2, Werner syndrome) in a first-degree relative, exposure to ionizing radiation and symptomatic nodule (e.g., rapid growth, hoarseness of voice, dysphagia. The aim of current study is to determine various risk factors towards malignancy using multiple logistic regression tests in which data encompassing population in Kelantan state.

CHAPTER 2:

LITERATURE REVIEW

2.1: Thyroid nodule

Thyroid nodule has become more prevalent worldwide due to increase awareness and development of new technology and medical equipment to detect other pathology that detects incidentalomas. The estimated annual incidence in United States is approximately 0.1% per year, equivalent to approximately 10% lifetime probability of developing thyroid nodule. They noted 4 times more common in women than man and incidence increasing linearly with age and low iodine intake. Hormonal influences was thought to be the main factor to this gender disparity, increasing nodule size and new nodule development mainly during pregnancy and multiparity. Another risk factor include history of exposure to ionizing radiation, either during childhood or occupational exposure (Popoveniuc and Jonklaas 2012).

In India, the prevalence of thyroid nodules ranges from 4% to 10% in the general adult population and from 0.2% to 1.2% in children. They are predominantly benign; only 5% to 30% are malignant (Manoj Gupta, Savita Gupta et al. 2010). In Taiwan it is reported that incidence of thyroid nodule is 19.4% in male, 33.6% in female and 25% in the total population and the incidence of malignancy is 5-10% (Chih-En Tseng, Chang-Kuo Wei et al. 2008). Whereas in Egypt, 4-7% of adult population was reported to have thyroid nodule with fewer than 5% are malignant (E.A. Sinna and Ezzat 2012).

A review by Htwe *et.al* noted the incidence of thyroid carcinoma among goitrous thyroid lesion based on different parts of Malaysia. The author concluded that higher incidence on male, papillary thyroid carcinoma is the most common type and suggested thyroid cancer arising from

multinodular goiter are high in iodine-deficient areas (Than 2012). It is this opportunity to validate another local data measuring the burden of thyroid nodules among our population that correlates the incidence towards malignancy.

2.2: Thyroid malignancy

2.2.1 The incidence of thyroid malignancy

Thyroid malignancy is rare and represents only 1% among all malignancies. According to a study done in year 2002, thyroid cancer incidence in Australia was published based on the data from Australia's regional cancer registries. There were 9,053 new cases of thyroid cancer in Australia for the period 1982-1997. Papillary, follicular, medullary, anaplastic, and "other diagnoses," accounted for 65.8%, 17.8%, 4.6%, 1.3%, and 10.5% of registered cases respectively (Burgess 2002). In France, data collected between the periods of 1978-1997 reported a total of 3853 adult cases of thyroid cancer. The incidence of thyroid cancer increased by 6.2% per year in men and by 8.1% per year in women (Colonna, Grosclaude et al. 2002). Similar report of increasing incidence of thyroid cancer was shown in an analysis by Hodgson *et al*. in Florida, United States between 1990 and 2000. The age-adjusted incidence rates increased from 4.2 per 100,000 to 7 per 100,000 in 2000 with estimated annual percent change of 5.5% ($p< 0.001$) in this period (Hodgson, Button et al. 2004).

Likewise, the incidence of incidental cancer in patients operated for benign thyroid disease was in the increasing trend as reported by a study conducted by Costamagna *et al*. The study included a total of 568 patients underwent surgery for benign thyroid disease. Incidental cancer was found in 53 patients (9.3%) in which 44 (83.0%) had papillary, 4 (7.5%) follicular carcinoma, 4 (7.5%) medullary carcinoma and 1 (1.9%) had primitive thyroid paraganglioma

(Costamagna, Pagano et al. 2013). The study carried out by Miccoli *et al*. revealed that a 10.4% of patients operated for benign thyroid disease had incidental thyroid malignancy from the final histopathology report (Miccoli, Minuto et al. 2006).

In Malaysia, the latest reported incidence of thyroid cancer has been published in year 2012 by Htwe *et al.* It was a review of hospital-based retrospective studies of thyroid cases done in Malaysia's hospital. Study periods ranged from three to 11 years. They noted a high incidence of goiter in seven states in Malaysia, higher among females aged 21-60. Papillary thyroid cancer is more common histological type compare to follicular cancer (Than 2012).

2.2.2: Thyroid malignancy in solitary thyroid nodule (STN) vs multinodular goitre (MNG)

The significant difference in the prevalence of thyroid cancer in thyroid glands with STN versus MNG remains uncertain until date. Most of the studies showed more prevalence of thyroid cancer in STN compare to MNG. A meta-analysis was performed to evaluate the comparative prevalence of thyroid cancer in STN and MNG. Fourteen studies were recruited in the study. It encompassed 23565 patients with MNG and 20723 patients with STN. MNGs were found to have a lower risk of thyroid cancer than STN with OR 0.8 (0.67,0.96) (Brito, Yarur et al. 2013).

A study done comparing malignancy in a nonfunctioning STN and MNG reported that malignant involvement in cold nodules of MNG did not differ significantly from that found in STN. In this study, thyroid scans were performed for all the subjects. FNAC was performed for all cold nodules in 146 patients and thyroid cancers were confirmed by final pathology report after surgery. The rate of malignancy in cold nodules in the MNG group was 9.78%, in comparison with 8% in the group with STN ($p = 0.89$). It was concluded that no significant

difference in the prevalence of malignancy in cold nodules in MNG and STN (Sachmechi, Miller et al. 2000). Gandolfi *et al*. and Pradhan *et al*. both reported a significant percentage of thyroid cancer in MNG which was 13.7% and 13.6%, respectively (Gandolfi, Frisina et al. 2004, Pradhan, Shrestha et al. 2011).

2.3: Preoperative diagnosis of thyroid malignancy

2.3.1: Thyroid Ultrasonography

Diagnostic thyroid ultrasonography should be performed in all patients with a suspected thyroid nodule. It is important technique used in the detection and evaluation in the settings of noninvasiveness, inexpensive procedure and the information that can be retrieved. Real-time, 2-dimensional and high resolution USG has become widely accepted standard of care in accurate measurement, ability to characterize nodules, subclinical nodule identification or impalpable nodule and utility in characterization and mapping of pathological lymph nodes (Bryan McIver 2013).

Previous study has investigated the ability of USG to differentiate between benign and malignant nodule and have found independent risk factors for malignancy such as microcalcifications, irregular or microlobulated margins, hypoechogenicity, taller-than-wide shape and increase intranodular vascularity (Popoveniuc and Jonklaas 2012). However it is not sufficient to conclude a lesion without these suspicious features is benign because their positive predictive value is lowered by their relatively low sensitivity, but a combination of at least 2 of them is pointing towards malignancy (Enrico Papini, Rinaldo Guglielmi et al. 2002). Presence and identifications of cervical lymph nodes showing microcalcifications, increased vascularity, cystic changes and rounded shape with alongside ipsilateral thyroid nodule are important features

that suggest malignancy. In addition, evidence of extracapsular growth with perithyroidal muscle infiltration and recurrent laryngeal nerve extension is another indicator pointing towards malignancy (Popoveniuc and Jonklaas 2012).

Several advanced USG techniques have been evaluated to increase the value in diagnosing malignant nodules. Doppler technique or assessment of blood flow has been incorporated into ATA guidelines. Elastography a technique that assess the stiffness of a nodule through measurement of compression USG has shown promise to give more information towards malignant lesions. This technique was demonstrated to be highly specific (96%-100%) and sensitive (82%-97%) in the diagnostic evaluation of thyroid nodules (Elniel H. Eltyib, A. Awad et al. 2014).

2.3.2: Fine needle aspiration cytology

Fine needle aspiration biopsy or cytology has an established role in the evaluation of euthyroid patients with a thyroid nodule. It is proven to reduce the rate of unnecessary thyroid surgery for patients with benign nodules and appropriately triages patients with thyroid cancer to appropriate surgery. Before the implementation of thyroid FNAC routinely, the percentage of surgically resected thyroid nodules were malignant was 14%, whereas it has surpasses 50% with the current practice (Edmund S. Cibas and Syed Z. Ali 2009). In this study, we would like to demonstrate the ability of FNAC in determining malignancy in thyroid nodules.

Traditionally accepted that false negative FNAC remains as low as 5% in detecting malignancy, however there are reported studies that showed the specificity and sensitivity of FNAC may varies due to several reasons. Chih et.al reported 19% false negative rate due to; i) incidental findings or papillary microcarcinoma, cytologic sampling error and underdiagnosis

(Chih-En Tseng, Chang-Kuo Wei et al. 2008). Morgan et.al reported higher false negative rate of FNAC of 45.8%. They noted that only at second aspirates showed malignant changes in operated nodule. Thus they recommend if solitary thyroid nodule is to be observed, a second aspirate should be undertaken to confirm it is truly negative for malignancy (Joanna L. Morgan, Jonathan W. Serpell et al. 2003). A multicenter prospective analysis done by Chung et.al agreed that the rate of false positive and false negative of FNAC still remains challenging. Their view on development of molecular diagnostic test would offer more beneficial in the future in the diagnosis of thyroid nodule (Chung-Che Charles Wang, Lyssa Friedman et al. 2011).

FNA under ultrasound guided is currently the preferred way over palpation-guided approach in view of lower rates of false negative and non-diagnostic cytology, mainly in thyroid nodule that are non-palpable, deeply located and predominantly cystic component (Popoveniuc and Jonklaas 2012).

2.3.3: Serum markers

Many studies done had supported the relationship between high preoperative TSH levels and thyroid cancer in nodular thyroid disease. Carles Zafon *et. al* has demonstrated that patients with malignancy had higher median values of TSH than did patients with benign nodules (1.62mU/L (0.9-2.5) versus 0.9mU/L (0.3-1.6), respectively; p value < 0.001). The ROC analysis also showed a TSH cut off point of 1.08mU/L to differentiate benign from malignant with sensitivity of 72% and specificity of 57% and with area under the curve (AUC) of 0.67 (Zafón, Obiols et al. 2014).

Another serum marker that can be considered is serum calcitonin. It is a sensitive marker for detection of C-cell hyperplasia and medullary thyroid cancer (MTC) (Popoveniuc and

Jonklaas 2012). Because of the rarity of the entity, thus screening with this marker is not widely recognized. However in the setting of surveillance and prognosis of MTC it does play a role.

2.4: Associated risk for malignancy in thyroid nodule

It has been well studied and recognized predictors of malignancy in patients with thyroid nodules. Various parameters include age younger than 20 or older than 70, male gender, large nodule size (>4cm), rapid growth, associated hoarseness, dysphagia or lymphadenopathy, history of exposure to radiation to head and neck, which particularly in childhood and type of goiter (single nodule versus multinodular goiter) (M. Regina Castro, Rachel P. Espiritu et al. 2011). Sonographic evidences that have been found to be indicative of malignant potential are also well described. Microcalcifications, irregular or microlobulated margins, hypoechogenicity, taller-than-wide shape and increased intranodular vascularity (Popoveniuc and Jonklaas 2012).

In this study, we try to elicit general epidemiology and thyroid nodule status and the analysis will be made through which nodule are malignant using various statistical method. In a reported study, they noted that the risk of malignancy in a thyroid nodule increases with serum TSH concentrations with the value is within normal range (K. Boelaert, J. Horacek et al. 2006, Zafón, Obiols et al. 2014). Using binary logistic regression analysis, they revealed significantly increase adjusted odds ratios (AORs) for the diagnosis of malignancy in subject with higher serum TSH compare to those with TSH level of 0.4mU/liter (AOR 2.72, 95% CI 1.02-7.27, P = 0.046) (K. Boelaert, J. Horacek et al. 2006).

Another study which was done in Spain looking for risk factors for malignancy in multinodular goiters found that by using multivariate analysis, they noted that family history of

thyroid pathology, personal history of cervical radiation therapy, recurrent goitre and presence of cervical lymphadenopathy (A. Rı´osa, J.M. Rodrı´gueza et al. 2004).

Since the introduction of Bethesda classification for reporting of thyroid cytopathology, many literatures have look into more detailed risk factors for thyroid nodule which classified as Bethesda category III (Atypia of undeterminate significant (AUS)/ follicular lesion of undeterminate significant (FLUS)) to become malignant. Approximately 10% of of thyroid nodule are classified as Bethesda III that warrants surgical excision and among many reported series the prevalence of malignancy among this group ranging from 5% to 37% and Yolanda et.al noted in their study that 42.9%-48% on patients with indeterminate thyroid nodule cytopathology turned out to be malignant (Vazquez, Alvarez et al. 2015).

Kirtee et.al noted male patients with cytology AUS/FLUS were more frequent to have malignant tumours and larger nodule size of more than 2cm or larger was significantly a risk factor (Kirtee Raparia, Soo Kee Min et al. 2009). Another study proposed the similar risk factors that showed higher risk towards malignancy which are male and thyroid nodule size of more than 4cm (Trimboli, Treglia et al. 2013).

Kuru *et.al* reported from multivariate logistic regression analysis on their data revealed solid structure, microcalcification, hypoechogenicity, increased vascularization and irregular margin were found to be significant and independent risk factors for malignancy (B. Kuru , Atmaca et al. 2015). Jun *et.al* reported concluded male gender, microcalcification and lymphadenopathy were independent risk factors for malignancy in their record review (Jun D. Tai, Jin L. Yang et al. 2012).

CHAPTER 3:

OBJECTIVES

3.1 General objectives

To study the agreement between cytopathology and histopathology and the risk factors of malignancy in patients with suspicious thyroid nodule who underwent thyroidectomy.

3.2 Specific objectives

1. To determine the prevalence of thyroid malignancy in patients with thyroid nodule.
2. To determine the agreement between cytopathology and histopathology of thyroid nodule.
3. To determine sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value of FNAC in thyroid nodule
4. To identify the associated factors contributing to malignant histopathology in suspicious nodule.

3.3 Research Questions

1. What is the prevalence of thyroid malignancy in patients with thyroid nodule?
2. Is there an agreement between cytopathology and histopathology of thyroid nodule in thyroidectomy patients that can be identified?
3. What are the main risks factor that contributing to malignant diagnosis in suspicious nodule?
4. What are the sensitivity, specificity, diagnostic accuracy, positive predictive value and negative predictive value of FNAC in thyroid nodule?

CHAPTER 4:

MATERIALS AND METHODS

4.1 Study design and source population

This is a retrospective record review study of patients who had undergone thyroidectomy in the Department of Breast and Endocrine Surgery, Hospital Raja Perempuan Zainab II (HRPZ II), Kelantan, Malaysia from June 2008 to May 2013. Recruitment of subjects was done based on the record of the list of patients who underwent thyroidectomy. The data of patients was retrieved from patients' medical records.

4.2 Eligible subjects

All patients who fulfilled the inclusion and exclusion criteria were recruited

Inclusion criteria:

- All patients who have undergone thyroidectomy with preoperative cytology within duration of study period.

Exclusion criteria:

- Patients underwent completion thyroidectomy with prior history of surgery for thyroid cancer (only the first surgery will be recruited).
- Patient that underwent cervical nodal biopsy prior to surgery.
- Incomplete documentation of patient's case note.

4.3 Sample size

Sample size calculation was done using various methods according to each objective. For the first objective, Sample size Sample Size Calculation v1.7.1 by Dr. Wan Nor Arifin, which is available at medic.usm.my has been used. The calculation was to achieve a 95% confidence interval of absolute precision +/- 5% with 5% level of significant. 10% non-respondents was expected and added to achieve the final estimated sample size.

Estimated sample size for second objective has been calculated using Kappa agreement test. The power of study was set at 80% and α value was 0.05, and result been added with 10% drop out. The Sensitivity and specificity test has been used to calculate estimated sample size for objective 3. Corrected sample size that has been calculated was 273. The fourth objective was estimated by using Multiple logistic regression test, calculated Using G*Power v3.1.9.2.

(See detail sample size calculation in Appendix A)

4.4 Research protocol

The study protocol was reviewed and approved by the Human Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia (Appendix B) as well as the Medical Review and Ethics Committee, Ministry of Health Malaysia (Appendix C). A list of patients who underwent thyroidectomy from June 2008 through May 2013 was obtained from the record in the operation theater. Patients who fulfilled the inclusion and exclusion criteria were recruited in the study. Their medical records were reviewed retrospectively.

In HRPZ II, patients who were indicated and agreed for surgery were scheduled for elective surgery. Occasionally, emergency thyroidectomies were performed for patients with

obstructive goiter that required ventilation. For elective cases, a routine history taking and physical examination were performed to obtain basic demographic data, to assess the presence of pressure symptom, to check the status of thyroid function and to examine the thyroid enlargement. Biochemical test for thyroid function was taken for each patient routinely, and presence of hyperthyroidism or hypothyroidism was treated to ensure euthyroidism at the time of surgery. Pre-operative ultrasonography and FNAC were performed only for those who indicated. FNAC were performed without ultrasound-guided in HRPZ II. Reporting of FNAC result will be reviewed within patients' medical record.

The final histopathology will be reviewed and analyzed. The proportion of thyroid nodule with suspicious cytopathology will be compared with final histopathology of the sample. The associated factors for thyroid malignancy in this group were analyzed. The variables included in the study were age and sex of patients, duration of nodule, presence of pressure symptoms, thyroid function status, nodularity and site of the thyroid gland, FNAC and HPE findings and type of surgery. There was no follow up done to look for surgical complications after the surgery for this study.

4.5 Data collection

Permission from the Director of HRPZ II for retrieving the patients' medical records from record office was officially granted prior to the data collection activities (Appendix D). Data collected was recorded in a pre-formatted data collection form (Appendix E). The data recorded include patients' demographic data (Age, sex and ethnics), pre-operative information (pressure symptoms, nodularity of goiter, thyroid function status and FNAC result), operative information (date and duration of procedure, indication for surgery, type of surgical procedure, seniority of the surgeon, the weight of resected specimen and intra-operative surgical complication), post-operative surgical complication (temporary hypocalcaemia, permanent hypoparathyroidism, recurrent laryngeal nerve palsy, haemorrhage, surgical site infections, other organ injury and death), and final histopathological diagnosis after surgery.

4.6 Data analysis

Data entry and analysis was done using Statistical Package for Social Science (SPSS) version 22.0. For descriptive analysis, categorical variables were expressed as frequencies (n) and percentages (%) while numerical variables were reported as mean with standard deviations (SD). The proportions of thyroid nodule and the proportions of thyroid malignancy were described as frequencies (n) and percentages (%).

In order to determine the agreement between cytopathology and histopathology of thyroid nodule, Cohen's Kappa test was implemented and the method of calculation is based on Clinical Agreement by Dr Wan Nor Ariffin, Unit of Biostatistics and Research Methodology, Universiti Sains Malaysia. wnarifin@usm.my.

		Histopathology		
		Malignant	Non malignant	Total
Cytopathology	Malignant	72	23	95
	Non malignant	9	229	238
	Total	81	252	333

Kappa, κ = observed proportion of agreement – expected proportion of agreement by chance

1-Expected proportion of agreement by chance

$$= \frac{p_0 - p_e}{1 - p_e}$$

$$P_0 = \frac{\text{sum of observed agreement}}{\text{Total}} = \frac{\sum fii}{n}$$

$$P_e = \frac{\text{sum of expected agreement by chance}}{\text{Total}} = \frac{\sum rici/n}{n}$$

Standard error and confidence interval for κ

- Standard error and κ is given by

$$SE\ (\kappa) = \sqrt{\frac{p0\ (1-p)}{n(1-pe)2}}$$

- Confidence interval of κ is given by

$$\kappa \pm z_{\left(1-\frac{\alpha}{2}\right)}\ x\ SE(\kappa)$$

The demographic (age, sex, duration) and clinical (nodularity, pressure symptoms, thyroid function status) variables were analyzed as the associated factors for thyroid malignancy in large goiter. In univariable analysis, independent variables were initially assessed individually using the Simple Logistic Regression to determine the significant of each variable in predicting its association with thyroid malignancy. Odds Ratio (OR) and respective 95% Confidence Intervals (CI) were calculated for all clinical parameters. In multivariable analysis, Multiple Logistic Regression was applied. Preliminary main effect model was created with Forward Likelihood Ratio (LR) on all the clinical important and significant variables. Multicollinearity and interaction were checked to obtain the preliminary final model. The final model was checked for fitness using the Hosmer-Lemesshow test, classification table to count for overall correctly classified percentage, and the Receiver Operating Characteristic (ROC) curve to check for the area under ROC curve. The adjusted Odds Ratio was obtained from the final model. The level of statistical significance was set at $p < 0.05$.

4.7 Methodology Flow Chart

Source population was identified.

Patients underwent thyroidectomy in HRPZ II from June 2008 till May 2013

(5 years duration)

Cases were identified from the record in the operation theater.

Inclusion criteria:

- All patients who have undergone thyroidectomy with preoperative cytology within duration of study period.

Recruited

Study subjects

Data collection:

Demographic and clinical data

TFT and FNAC results

Histopathological reports

Complete Data

Data Analysis

Results

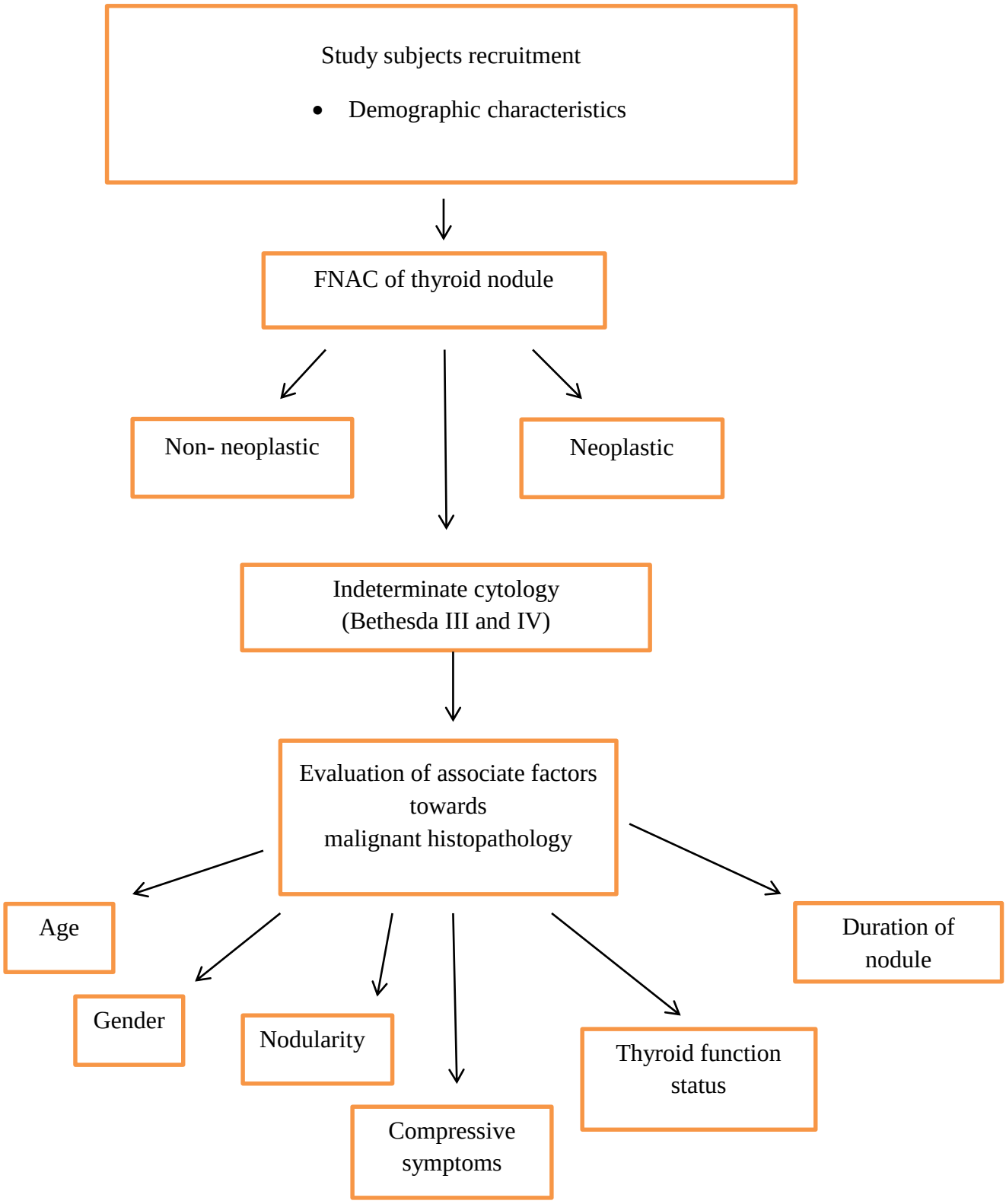
Exclusion criteria:

- Patients underwent completion thyroidectomy with prior history of surgery for thyroid cancer (only the first surgery will be recruited).
- Patient that underwent cervical nodal biopsy prior to surgery.
- No FNAC done prior to surgery.
- Incomplete documentation of patient's case note.

Excluded

Incomplete data

4.8 Frame work



CHAPTER 5:

RESULTS

There were five hundred and sixty one patients who underwent thyroid surgery throughout the study period from June 2008 to May 2013. Total of 185 patients were excluded from the study in view of lost records or incomplete data. Out of 481 patients with sufficient data, only 333 patients fulfilled the inclusion and exclusion criteria. The number of total subjects that involved in this study fulfilled the calculated sample size requirement. Total number of 148 excluded from the study due to various reasons; patients who underwent completion thyroidectomy due to malignant histopathology from previous surgery and patients that underwent cervical nodes biopsy prior to surgery.

5.1 Description of demographic data among thyroidectomy patients.

A total of 333 subjects were recruited during the study period. The mean (SD) age was 44.07 (14.33) years and 54 (16.22%) were male (as shown in Figure 5.1.1& 5.1.2). The occurrence of thyroid nodule is dominated by female in 1:4 ratios. Majority of the subjects are from Malay ethnic (94.6%) (Figure 5.1.3) and the mean chronicity of nodules is 4.72 years (Figure 5.1.4). In our series, the right thyroid nodule is more dominant than left side encompassing 60.7% and multinodularity accounts for 51.1%.

FNAC results were interpreted as benign in 210 cases (63.1%), AUS or follicular lesion of undetermined significance in 20 cases (6.0%), follicular neoplasm or suspicious follicular neoplasm in 45 cases (13.5%), suspicious for malignancy in 17 cases (5.1%), malignant in 33 cases (9.9%) and non-diagnostic or unsatisfactory in 8 cases (2.4%), as shown in Table 5.1.1.