

Expression of CD56 in Follicular Patterned Thyroid Neoplasms

*Thesis
submitted for partial fulfilment of
M.Sc. degree in Pathology*

By
Ahmed AbdulAleem AbdulRahman Hussein
MBBCh

Under Supervision of

Prof. Dr. Samira Abdullah Mahmoud
Professor of Pathology
Faculty of medicine, Cairo University

Dr. Rasha Ahmed Khairy
Lecturer of Pathology
Faculty of medicine, Cairo University

Dr. Mohammed Mahmoud Abdellah
Lecturer of Pathology
Faculty of medicine, Fayoum University

**Faculty of Medicine
Cairo University
2016**

Abstract

The current study included 45 cases of thyroid follicular lesions; 15 cases of follicular variant of papillary thyroid carcinoma, 20 cases of follicular adenoma, and 10 cases of follicular carcinoma. All cases were retrieved as routinely processed paraffin blocks taken from Kasr AlAiny and other labs, dating between 2014 and 2016. For all specimens, some clinical data were available on the computer files including age and sex of the patients and the staging of the tumor. All the cases included in our study were thyroidectomy specimens.

Two sections were prepared from each paraffin block to be studied both histologically (by Hematoxylin and Eosin stain) and immunohistochemically for CD56 expression by using [Monoclonal Mouse Anti-Human CD56 (Dako, Clone 123C3)]. Immunostaining was done by using Autostainer Link 48 (Dako). Cases were considered positive when more than 10% of the cells in the examined lesion were stained positively by CD56.

Key words:

Thyroid – Neoplasms – CD56 – Follicular Pattern

AKNOWLEDGMENT

I am deeply indebted to Prof. Dr Samira Abdullah Mahmoud, Professor of Pathology Cairo University, for her valuable and fruitful suggestions, continuous encouragement and great effort in overcoming all obstacles that faced this work.

I am most grateful to Prof. Dr. Rasha Ahmed Khairy, Professor of Pathology Cairo University, for her tutelary supervision and kind guidance, her valuable advice and continuous encouragement has helped me a lot in completing this work.

I would like to sincerely thank Prof. Dr. Mohammed Mahmoud Abdullah, Assistant Professor of Pathology Cairo University, for his encouragement, real help and positive attitude all through this work.

I would like to thank my father, mother and my wife; without their love and support, this work would have never seen light.

CONTENTS

Introduction_____	1
Aim of the Work_____	3
Review of the Literature_____	4
• Development and Embryology_____	4
• Anatomy_____	5
• Histology_____	6
• Immunohistochemistry_____	8
• WHO Classification of Thyroid Tumors_____	9
• Follicular Adenoma_____	10
• Thyroid Carcinoma_____	16
• Papillary Thyroid Carcinoma_____	18
• Follicular Thyroid Carcinoma_____	37
• Oncocytic (Hurthle Cell) Tumors_____	41
• Medullary Carcinoma_____	45
• Rare Thyroid Carcinomas_____	50
• Differential Diagnosis of Thyroid Lesions with Follicular Pattern_____	56
• CD56_____	62
• TNM Staging of Thyroid Tumors_____	64
Materials and Methods_____	66
Results_____	68
Discussion_____	82
Summary_____	89
Conclusion and Recommendations_____	91
References_____	92
Arabic Summary	

LIST OF TABLES

Table (1): CD56 expression significance in both Papillary Carcinoma and Follicular Adenoma_____	73
Table (2): CD56 expression significance in both Papillary Carcinoma and Follicular Carcinoma_____	74
Table (3): CD56 expression significance in both Follicular carcinoma and Follicular Adenoma_____	74
Table (4): Sensitivity and Specificity of follicular adenoma, papillary carcinoma, and follicular carcinoma for CD56_____	75
Table (5): Positive predictive value (PPV) and negative predictive value (NPV) of follicular adenoma, papillary carcinoma, and follicular carcinoma for CD56_____	75

LIST OF GRAPHS

Graph (1): Age distribution in thyroid lesions encountered in the present study_____	70
Graph (2): Sex distribution in thyroid lesions encountered in the present study_____	71
Graph (3): Stages of the 15 cases of papillary thyroid carcinoma and the 10 cases of follicular thyroid carcinoma_____	72

LIST OF CHARTS

Chart (1): Distribution of thyroid lesions encountered in the present study_____	68
---	-----------

LIST OF FIGURES

- Figure (1):** Papillary thyroid carcinoma, follicular variant_____76
- Figure (2):** Follicular carcinoma, showing capsular invasion____76
- Figure (3):** Follicular carcinoma, showing capsular invasion____77
- Figure (4):** Follicular adenoma with microfollicular and normofollicular patterns of growth_____77
- Figure (5):** The peripheral nerves in the musculosa of the colon__78
- Figure (6):** Papillary thyroid carcinoma, follicular variant, negative for CD56_____78
- Figure (7):** Papillary thyroid carcinoma, follicular variant, negative for CD56_____79
- Figure (8):** Papillary thyroid carcinoma, follicular variant, normal adjacent thyroid follicles, positive for CD56_____79
- Figure (9):** Follicular thyroid carcinoma, positive for CD56____80
- Figure (10):** Follicular thyroid carcinoma, positive for CD56____80
- Figure (11):** Follicular thyroid adenoma, positive for CD56____81
- Figure (12):** Follicular thyroid adenoma, positive for CD56____81

Introduction

Papillary carcinoma is the most common type of thyroid malignancy. Females are more affected than males. It can present in any age group, the mean age at the time of initial diagnosis being approximately 40 years (**Rosai and Tallini, 2011**). Its incidence in absolute numbers and in proportion to other thyroid tumors has been steadily increasing in the United States and many other countries around the world (**Nikiforov and Otori, 2012**).

Papillary carcinoma may sometimes be seen in a familial setting. Other potential risk factors include goiter, thyroiditis, history of prior exposure to radiation, and Graves' disease. Somatic rearrangements of the *ret* protooncogene which is located on the long arm of chromosome 10 and encodes a membrane –associated tyrosine kinase receptor have been seen in papillary thyroid carcinoma (**Khan and Nosé, 2010**).

Papillary carcinoma usually presents as a thyroid mass/nodule. Occasionally, patients with papillary thyroid carcinoma present with cervical lymphadenopathy. Tumors can range from less than a centimeter to several centimeters in diameter. They usually show a firm tan-white cut surface with irregular borders and occasional gross deposits of calcification (**Khanafshar and Lloyd, 2011**).

The diagnosis of papillary carcinoma is based on the tumor's characteristic nuclear features that need to be seen in a significant population of cells. The nuclei in papillary thyroid carcinoma show overlapping and loss of polarity, powdery to clear chromatin "Orphan

Annie eye nuclei,” intranuclear grooves, and occasional pseudoinclusions (**Khanafshar and Lloyd, 2011**).

While the characteristic nuclear features described above are common to all histological types of papillary carcinoma, a number of variants have been described on the basis of either size or architectural patterns of the tumor. Follicular variant of papillary thyroid carcinoma is the most common variant and is also the one that has generated a lot of controversy in its diagnosis (**Khan and Nosé, 2010**).

The follicular variant of papillary carcinoma manifests overlapping histopathological features with other follicular-patterned thyroid neoplasms (follicular adenoma, follicular carcinoma), and thus, not infrequently, poses a diagnostic challenge for pathologists. Multiple studies have demonstrated great inter-observer and intra-observer variability in histopathological diagnosis of follicular pattern thyroid cancer. This variability is because agreement about the minimal criteria required for changes in nuclear features of papillary thyroid cancer, as well as the exact criteria for capsular invasion, are not universally accepted. Therefore, the search for objective markers of thyroid malignancy is of great importance (**Paunovic et al., 2012**).

Many immunohistochemical markers were suggested for the differential diagnosis of these lesions. CD56 is one of these markers. Lack of CD56 expression was shown to be sensitive and specific to papillary carcinoma (**El Demellawy D et al., 2008**). CD56 (also called neural cell adhesion molecule (N-CAM)) is a membrane glycoprotein expressed on neural and muscle tissues (**Lanier et al., 1989**). Loss of expression of neural cell-adhesion molecule (N-CAM) is implicated in the progression of tumor metastasis (**Cavallaro et al., 2001**).

Aim of the Work

It is a histopathological and immunohistochemical study to differentiate between follicular variant of papillary thyroid carcinoma and other follicular-patterned thyroid neoplasms (follicular adenoma and follicular carcinoma) using anti-CD56 antibodies.

Development and Embryology

The thyroid gland develops from the larger median anlage and the two lateral anlagen. The medial anlage, which forms the major portion of the thyroid, is derived from the floor of the foregut and the two lateral anlagen are derived from the endoderm of fourth and fifth branchial pouches like the ultimobranchial bodies. The medial anlage appears by day 24 as median endodermal diverticulum from the base of the tongue in the region of foramen cecum. The diverticulum descends down from the foramen cecum into the neck along the midline attached to the thyroglossal duct. It reaches its final position anterior to the trachea by about 7 weeks; it then grows laterally and becomes bilobed (**McNicol and Lewis, 1996**).

Aberrations in the descent of thyroid may lead to persistence of thyroid tissue anywhere along the path of the thyroglossal duct including posterior tongue, midline of the neck and the anterior mediastinum. As mentioned below, thyroid lesions including tumors may arise in any of these aberrant locations, which should be considered when investigating for thyroid disorders. Early during the fifth week, the thyroglossal duct loses its lumen and shortly afterward breaks into fragments (**Organ et al., 2000**). However, the caudal end of the thyroglossal duct may persist in some embryos and this constitutes the pyramidal process, which is present in about 75% of mature human thyroids (**Murray, 1998**).

The lateral thyroid anlage becomes attached to the posterior surface of the thyroid during the fifth week and contributes up to 30% to the thyroid weight (**Organ et al., 2000**). The causes of the fusion of the lateral and medial anlage are unknown. It is speculated that migration of

the ultimobranchial body controls the growth of the medial anlage or that the growth of the medial anlage laterally and caudally inhibits expansion of the ultimobranchial body (**Organ et al., 2000**). The lateral thyroid anlage is thought to give rise to the calcitonin producing C cells and the solid cell nests. It is believed that the C cells are derived from the neural crest; they migrate to the ultimobranchial body and are subsequently incorporated into the thyroid (**Lloyd et al., 2002**). However, the existence of mixed follicular and C cell tumors raises the possibility of the common stem cell origin for both follicular and C cells, as is seen in the gastrointestinal tract (**McNicol and Lewis, 1996**).

The thyroid gland initially consists of a solid mass of endodermal cells, but small groups of epithelial cells are soon identified. The first follicles form epithelial plates at the beginning of eighth week and by twelfth week the plates are entirely converted into follicles. The development of the human fetal thyroid has been divided into three stages by LiVolsi to include precolloid stage (7–13 weeks); colloid stage (13–14 weeks); and follicular stage (after 14 weeks) (**Livolsi, 1990**). Evidence of thyroxin comes with the appearance of colloid and T4 and TSH are detectable in the circulation of human fetuses after 12 weeks (**Murray, 1998 and Organ et al., 2000**).

Anatomy

The thyroid is normally located in the mid portion of the neck anterior to the trachea and larynx, just below the cricoid cartilage attached by a loose connective tissue capsule. The recurrent laryngeal nerves lie in the groove between the lateral lobes and the trachea. The superior and inferior parathyroid glands are found close to the posterior surface of the gland or they may be located within the gland itself. The thyroid gland

consists of two lobes joined by an isthmus. In adults, the two lobes measure about 2–2.5 cm wide and 4–5 cm long. In some glands a pyramidal lobe, derived from the distal portion of the thyroglossal duct, extends upward from the isthmus (**Khan and Prasad, 2009**).

At birth the thyroid weighs 1–2 g and it increases to 10–15 g at puberty (**Kay et al., 1966**). In the adult the normal weight ranges from 15 to 35 g. The weight varies with iodine intake, sex, age, and functional status of the gland. In addition there are geographic variations ranging from an upper limit of up to 42 g in a Portuguese study (**Sobrinho-Simoes et al., 1979**) to about 10–20 g in North American population (**Mochizuki et al., 1963 and Pankow et al., 1985**). The mean weight is always higher in women and the weight varies in size with the menstrual cycle (**Hegedus et al., 1986**). In the elderly the weight may sometimes reduce to as little as 10 g (**McNicol and Lewis, 1996**).

Histology

On section, the thyroid gland has a brown cut surface and it is composed of multiple lobules separated by thin fibrous septa. Each lobule is made up of 20–40 follicles and is supplied by a single intralobular artery. The thyroid contains three major types of epithelial cells. These include follicular cells, which line the follicles and secrete thyroxine and triiodothyronine; C cells, which secrete calcitonin; and the solid cell nests (SCN) that are remnants of the ultimobranchial body. The follicles are filled with colloid, range in size from 50 to 500 micron (average 200 micron) in diameter and are lined by cuboidal to low columnar epithelium. The cells lining the follicles have a basal nucleus and rest on a basement membrane composed of laminin and collagen IV (**Kendall et al., 1985**).

The colloid includes thyroglobulin which is a glycoprotein giving it a PAS-positive diastase resistant staining characteristic. The amount of the colloid and the height of the follicular lining cells vary with the functional status of the gland. In a hyperactive state the follicles are lined by tall cells and have less amount of colloid. In addition to colloid the follicles contain birefringent calcium oxalate monohydrate crystals, the numbers of which may increase with age. This finding may be useful sometimes to differentiate between thyroid follicles and parathyroid tissue on frozen section evaluation **(Isotalo and Lloyd, 2002)**. Intracytoplasmic fat within thyroid follicular cells may be detected in 50% of glands on oil red O staining and this may increase with age **(McNicol and Lewis, 1996)**. Due to the intimate development of thyroid with the mesodermal structures of the neck, fat, cartilage, and/or muscle may be found within the thyroid capsule. For the same reason normal thyroid tissue may be found intermingled with the neck soft tissues including muscle, which should not be mistaken for metastatic carcinoma **(Livolsi, 1990)**.

The solid cell nests can be seen in up to 60% of thyroid glands in the mid portions of the lateral lobes **(Harach, 1988)**. They are solid irregular masses of epithelial cells measuring about 1 mm or less in maximum diameter and may be solitary or multiple, unilateral or bilateral. They are composed of polyhedral or oval cells with oval nuclei containing finely granular chromatin; nuclear grooves may be seen **(Lloyd et al., 2002)**. SCN may sometimes show cystic change and show positive staining reaction with acid mucins **(McNicol and Lewis, 1996)**.

In addition there are dendritic antigen presenting cells present in the parafollicular thyroid stroma; mast cells, and T lymphocytes may also be seen around the follicles **(Kabel et al., 1988)**.

Immunohistochemistry

On immunohistochemistry the follicular cells stain positively with low molecular weight cytokeratin, thyroglobulin, and thyroid transcription factor (TTF-1); vimentin co-expression may also be present. In situ hybridization studies have revealed thyroglobulin mRNA within the follicular cells (**Lloyd et al., 2002**). The C cells are intrafollicular and sometimes parafollicular in location and in normal glands are not visualized on routine hematoxylin and eosin (H&E) staining. However, they can be visualized readily on special histochemistry using a silver (Grimelius) stain and on immunohistochemistry by pan-neuroendocrine antibodies such as chromogranin and synaptophysin and specific antibodies including calcitonin and calcitonin gene related peptide (CGRP). In addition C cells stain positive for TTF-1 and may also express bombesin, somatostatin, gastrin-releasing peptide, low molecular weight cytokeratin, and CEA (**Lloyd et al., 2002**).

The SCN are positive for low molecular weight cytokeratin and CEA and show variable staining with calcitonin. The latter finding gives support to the theory of the SCN being derivatives of the ultimobranchial body (**Mizukami et al., 1994, Pianzola et al., 1995, McNicol and Lewis, 1996 and Lloyd et al., 2002**).

WHO Histological Classification of Thyroid Tumors

(Quoted from DeLellis et al., 2004)

Thyroid carcinomas:

Papillary carcinoma

Follicular carcinoma

Poorly differentiated carcinoma (insular carcinoma)

Undifferentiated (anaplastic) carcinoma.

Squamous cell carcinoma

Mucoepidermoid carcinoma

Sclerosing mucoepidermoid carcinoma with eosinophilia

Mucinous carcinoma

Medullary carcinoma

Mixed medullary and follicular carcinoma

Carcinoma showing thymus like differentiation

Thyroid adenomas and related tumors

Follicular adenoma

Hyalinizing trabecular tumor

Other thyroid tumors

Teratoma

Primary lymphoma and plasmacytoma

Ectopic thymoma

Angiosarcoma

Smooth muscle tumors

Peripheral nerve sheath tumors

Paraganglioma

Solitary fibrous tumor

Follicular dendritic cell tumor

Langerhans cell histiocytosis

Secondary tumors