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REVIEW ARTICLE

Comparative Analysis of Papillary Thyroid Carcinoma and Benign Lesions; Immunohistochemical Diagnostic Markers and Their Gene Expression Correlation.

Esraah Alharris[1], Samah Alkhalidy[2], Dina Saleh[1],
[1] Department of Pathology and Forensic Medicine, College of Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq..
[2] Histopathology and cytology unit, Laboratory division, Al-Diwaniyah Teaching Hospital. Al-Diwaniyah Health Office.Iraq,

*Corresponding author: E-mail: esraah.alharris@qu.edu.iq

Abstract:

Background:Migraine Papillary thyroid carcinoma (PTC) incidence has increased globally, but it is unclear if this was because of an improvement in the patient's knowledge or if the tumor was incidentally discovered. The microscopical features of the nuclei are the gold standard for diagnosing PTC. Many immunohistochemical markers have been used to accurately diagnose PTC, such as CK-19, TTF-1, HBME-1, ret, and S-100.

Objectives: To assess the incidence of PTC in the Al-Qadisiyah governorate and examine the expression of several markers by immunohistochemistry in thyroid tissue biopsies.

Materials and Methods: Two hundred and twenty surgically resected Thyroid glands were implanted in paraffin tissues after being treated in formalin. Then we use a hematoxylin and an eosin stain and immunohistochemical staining by TTF-1 and CK-19.

Results: Although benign lesions did not show TTF-1 and CK-19 staining, papillary thyroid carcinoma showed diffuse, strong staining. Furthermore, we used a free gene dataset to assess the genetic expression of genes encoding for TTF-1 and CK-19, TTF-1, and KRT-19 genes, respectively. Results showed that the TTF-1 gene is overexpressed in PTC cases as compared to benign lesions, while KRT-1 gene expression was not changed, which may indicate post-transcriptional KRT-19 gene regulation.

Conclusion: The immunohistochemical TTF-1 and CK19 staining could offer a helpful and objective means for confirming the diagnosis of challenging thyroid nodules. In addition, we identified that the TTF-1 protein upregulation is due to genetic overexpression of the TTF-1 gene, in contrast to CK-19, which was most likely post-transcriptionally upregulated.

Keywords: papillary thyroid carcinoma, cytokeratin, immunohistochemistry.

Introduction

The most common malignancy of thyroid follicular cells is papillary carcinoma (PTC) (1). Approximately 1% of all cancers are PTC, and almost 70-80% of all malignant thyroid tumors (2). Many predisposing factors were shown to predispose to the development of PTC, including radiation exposure, genetic mutations, and growth factors (3). If papillary thyroid carcinoma patients were treated early and appropriately, the long-term prognosis would be excellent. However, many of them may have metastasis in the lymph node at the time of diagnosis (4). Therefore, the pathologist's ability to provide a correct diagnosis is the primary factor determining the appropriate treatment (2). Previously, the presence of papillary architecture was the diagnostic feature for papillary thyroid cancer. Nowadays, diagnosis depends on the nuclear features represented by optically clear, elongated, overlapped micro-nucleoli, prominent nuclear grooves with irregular borders, and the presence of a pseudo-inclusion field (5). In many circumstances, detecting these features remains challenging, and it may be hard to distinguish between benign adenoma and papillary carcinoma. Cytokeratin-19 (CK-19) is a low molecular weight keratin and is one of the main proposed markers to confirm the diagnosis of papillary thyroid cancers and identify their subtypes (6). CK19 is encoded by the KRT19 gene, one of the dysregulated genes in PTC (7). It has been shown that papillary carcinoma of the thyroid shows a robust diffuse cytoplasmic immunohistochemical staining for CK-19 (8, 9). A diagnostic challenge may be faced when a follicular pattern of growth is seen within an encapsulated nodule showing empty nuclei with nuclear grooves or deeply staining colloid, and the distinction of NIFTP, or known as an encapsulated follicular variant of papillary carcinoma, from follicular adenoma will be hard (10). Furthermore, many other thyroid lesions may have papillary projections with focal nuclear features, such as multinodular goiter with delicate papillary budding and focal clear nuclear appearance, which could be misdiagnosed as PTC (11).

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The thyroid transcription factor gene (TTF1 or NKX) encodes for the TTF1 homeobox protein (12). Different tissues express the TTF1 gene, and the dysregulation results in many diseases, such as malignant tumors (13). The human variant of the TTF1 protein has 17 amino acids, which is described for the first time in thyroid follicular cells and later in other tissues such as the pulmonary tissue and the brain (14). It has been shown that peripheral lung sequencing is regulated by the TTF1 protein, which is named the terminal respiratory unit (TRU) (15). Various lung cancer stages show variable TTF1 immunohistochemical expressions (16). The TTF1 gene is expressed in around 70% of cases of pulmonary adenocarcinoma, especially cancers that possess a certain extent of TRU (17). In addition to lung adenocarcinoma, many brain lesions show mutations of the TTF1 gene, such as chorea and brain-lung-thyroid syndrome (18, 19). Although the expression of KRT-19 and TTF1 genes is closely related to the incidence of PTC, the main pathological changes at the molecular level are still unclear. In this paper, we showed the incidence of PTC in the Al-Qadisiyah governorate in Iraq compared to benign lesions, studied the immunohistochemical expression of diagnostic markers, and addressed the PTC genetic changes at the molecular level using the free online GEO.com website.

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METHODS (describes exactly what has been done; avoid detailed procedures when they are not created originally by the researcher; a reference citation is enough). Subtitles are enabled in this part (e.g., study design, sample selection, ethical consideration, outcome measurements, and statistical analysis):

The study was conducted in the Al-Diwaniyah Teaching Hospital in the Al-Qadisiyah governorate, Iraq, from 2019 to 2024. A total of 230 thyroid tissue samples embedded in paraffin were collected in the pathology department of the teaching hospital. Slides were obtained from the tissue blocks at 2.2-micron thickness, stained by hematoxylin and eosin (H&E stain). H&E-stained slides were re-examined blindly by four pathologists. The pathological diagnosis of PTC was verified and categorized according to the criteria of the WHO. The main cytologic features we depended on for the diagnosis of PTC were the clear nuclei, nuclear grooves, and crowded and overlapped nuclei. The samples were then collected and paraffin-embedded, sectioned at 4-micrometer thickness, and set for IHC staining with CK-19. The immunohistochemical staining was conducted using a mouse monoclonal anti-human antibody according to the manufacturer's instructions (DAKO Corporation, Glostrup, Denmark). For the negative control, the buffer was used to replace the primary antibody. The tissue sections of four-micrometer thickness were initially treated with pepsin for 10 minutes at room temperature. Endogenous peroxidase and nonspecific binding were then blocked. The primary antibody was then added after it was diluted at 1:100, followed by incubation for one hour at room temperature. The immunostaining was then detected using an ultra-streptavidin system (Signet, Dedham, MA).

The genetic analysis of PTC samples compared to normal thyroid tissue was obtained from the GEO website (a public, free online website that allows access to genetic data of different diseases).

RESULTS

Hematoxylin and eosin staining: Out of the 230 cases of thyroid samples studied, 141 cases (61%) were multinodular thyroid diseases, 16 cases (6%) were non-invasive follicular thyroid neoplasm with papillary-like nuclear features, 18 cases (7%) were Hashimoto's thyroiditis, 13 cases (5%) were follicular adenoma, six cases (2%) were oncocytic adenoma, three cases (1%) were Graves' disease, and 33 cases (14.3%) were papillary thyroid carcinoma, as shown in Fig. 1.

The diagnosis was made by examining slides stained with H&E stain and confirmed blindly by three pathologists, as shown in Fig. 2.

Immunohistochemical staining: The samples from normal thyroid tissue, benign tumors, and papillary thyroid carcinoma were assessed for CK-19 and TTF-1 immunohistochemical expression. The expression of CK-19 and TTF1 was positive in cases of papillary thyroid carcinoma, as shown in Fig. 3. C&D in comparison to benign and normal thyroid tissues, in which no expression has been noticed in Fig. 3A&B.

The immunohistochemical expression density was analyzed using ImageJ software online. There was a significant increase in the expression of CK-19 and TTF-1 in PTC samples as compared to control samples ($p = 0.0003$ and $p = 0.008$, respectively) as shown in Fig. 4.

Regarding genetic analysis: the GEO website was used to assess dysregulated genes, as shown in Fig. 5A & B. The mean difference and volcano plot, respectively, show the significantly different genes as colored dots; the blue ones are the down-regulated genes, while the red ones represent the up-regulated genes. Looking at the differentially expressed genes on the same website, we started to look at the genes encoding for TTF1 (NKX gene) and CK19 (KRT19 gene). We found that the KRT-19 gene was highly expressed in cases of PTC as compared to normal thyroid tissue (Fig. 5C&D). In contrast, we haven't seen any difference in the NKX-1 gene between the normal thyroid tissue and the PTC samples. (3.2.2).

DISCUSSION (Avoid inclusion of subjects related to the introduction; do not repeat the result details):

Study limitations (clarify the weak points of the study and suggestions for future work):

A complete normed linear space addressing Among the most prevalent malignant tumors is PTC, widely seen in the adolescent age group (20). To date, the primary mechanism responsible for the development of PTC is regarded as very complicated and poorly understood, even though the genetic factors contribute to its aetiology. Several researchers have proved that increased TTF1 and TTF2 gene expression advances PTC risk in the European population (21, 22). This study aimed to show the immunohistochemical expression of TTF1 and KRT19 in PTC tissues compared to normal tissue. Then, we assessed whether the protein expression was due to gene overexpression or post-transcriptional regulation using a free data repository known as the GEO dataset (https://www.ncbi.nlm.nih.gov/gds/?term=).

In this study, we identified that the immunohistochemical expression of TTF-1 was related to genetic overexpression of the TTF-1 gene. In contrast, CK-19 expression was not associated with KRT-19 gene overexpression, which may be attributed to post-transcriptional regulation.

Papillary carcinoma of the thyroid (PTC) is the most common malignant tumor of the follicular thyroid epithelium (23). The diagnosis of PTC, in most cases, relies on nuclear features such as clearing and the presence of nuclear grooves, large overlapped nuclei, and irregular nuclear outlines (5). In some instances, the differentiation between follicular adenoma and PTC is hard. Therefore, the immuno-staining with markers can be of great value in establishing the diagnosis (24). The recommended markers for thyroid malignancies are TTF-1 and CK-19, which are usually used to confirm PTC diagnosis (25).

In the present study, 220 samples of surgically removed thyroid tissue were used to assess the incidence of PTC in Al-Diwaniyah Governorate. We found that the incidence of PTC was 14.3% of thyroid, for which immunohistochemical studies showed overexpression of both CK-19 and TTF-1 in PTC cases as compared to benign thyroid lesions. The results were consistent with many previous studies done to evaluate their diagnostic accuracy in PTC diagnosis (25-28).

Although many researchers could have addressed our results before, few researchers looked at the correlation between the CK-19 and TTF-1 protein expression and their genetic changes. In this work, we have shown that the expression of TTF-1 protein was due to genetic upregulation; this finding was consistent with many researchers' conclusions (13, 29-31). On the other hand, some scientists showed that the TTF-1 gene may be expressed in normal and benign thyroid lesions (32, 33). Consistent with previous research, the expression of the KRT-19 gene was significantly higher in PTC compared to control and benign thyroid lesions (7, 34-36). However, our identification of higher TTF-1 protein expression in the absence of significant gene change suggests additional post-transcriptional gene regulation influencing the process of tumorigenesis.

This finding may improve our understanding of PTC tumorigenesis, enhance diagnostic accuracy, and develop targeted therapies.

A limitation of this study is the relatively small sample size used to assess the genetic expression of TTF-1 and KRT-19, which may limit the generalization of the findings. Additionally, the lack of such genetic information in different countries worldwide will prove its credentials.

Future studies must investigate the cellular pathways of such mutations in cases of PTC using an in vivo model. Moreover, exploring the interplay between the genetic and environmental factors could offer a comprehensive understanding of PTC pathogenesis.

In summary, this study showed the incidence of different thyroid lesions in Al-Diwaneyah City and the immunohistochemical expression of TTF-1 and KRT-19. In addition, our study provides valuable insight into the genetic landscape of PTC to pave the way for targeted therapeutic strategies and improve patient care.

Conclusion: The incidence of papillary carcinoma in Al-Qadisiyah governorate has been estimated to be 14.3% of all thyroid samples resected from patients who visited Al-Diwaniyah General Hospital from 2019 to 2024. The molecular basis behind the development of papillary carcinoma of the thyroid can be attributed to both pre- and post-transcriptional regulation.

Conflict of interest: The authors clarify that the study was performed without conflict of interest.

Statistical analysis of data

The immunohistochemical expression of CK-19 and TTF-1 was analyzed using a 2-sample t-test method as described in (38). $P < 0.05$ was regarded as statistically significant. GraphPrism software was used to analyze the data.

ABBREVIATION

PTC: Papillary thyroid carcinoma
CK: cytokeratin
TTF-1: thyroid transcription factor

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Figure legend

Figure.1. Bar chart showing the percentage of different thyroid lesions in total of the 230 cases of thyroid samples studied

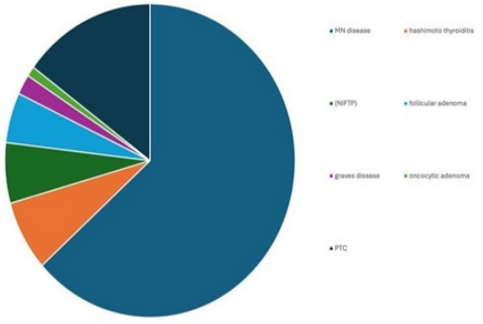
Figure.2. Haematoxylin and eosin staining was done to confirm the diagnosis of various thyroid lesions. A. Normal thyroid tissue. B. Follicular adenoma C. NIFTP (a non-invasive follicular variant of papillary thyroid carcinoma D. papillary thyroid carcinoma.

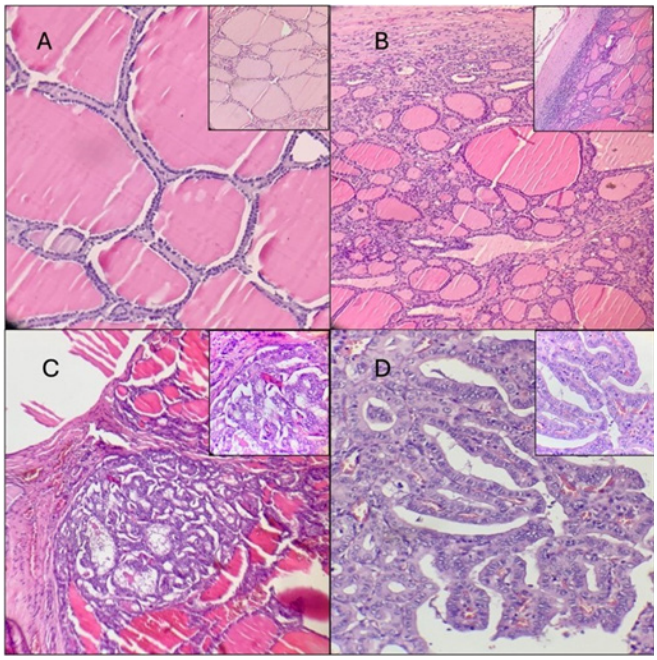
Figure.3 The immunohistochemical expression of CK-19 and TTF-1 in normal thyroid tissue, A.B., respectively. C. Expression of TTF-1 in PTC. D. Expression of CK-19 in PTC.

Figure.4. The integrated density of CK-19 and TTF-1 expression is significantly higher in PTC tissues than in control samples. The data was generated using ImageJ software. Significance ($p < 0.05$) for all the experiments was determined using an unpaired t-test.

Figure.5. The gene expression in cases of PTC as compared to normal thyroid tissue imported from the GSE website.

Tables and Figures

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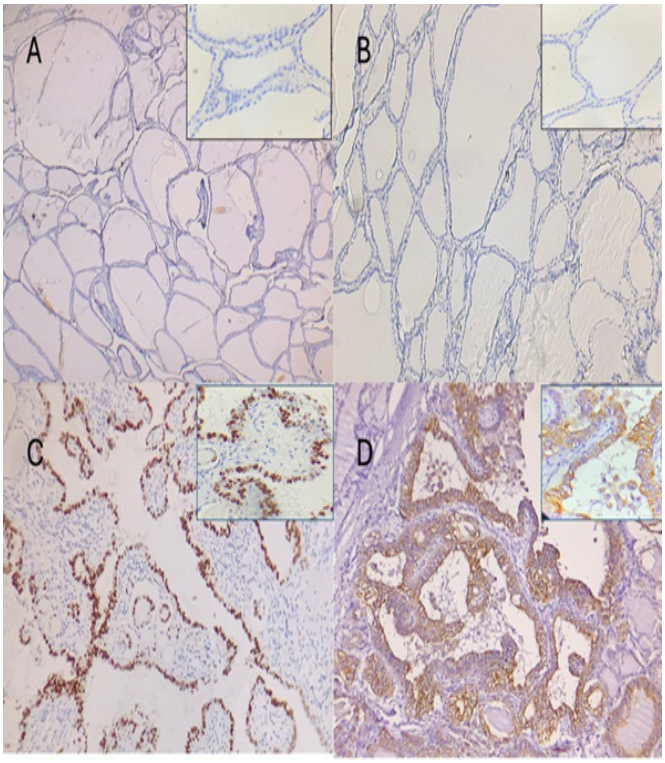
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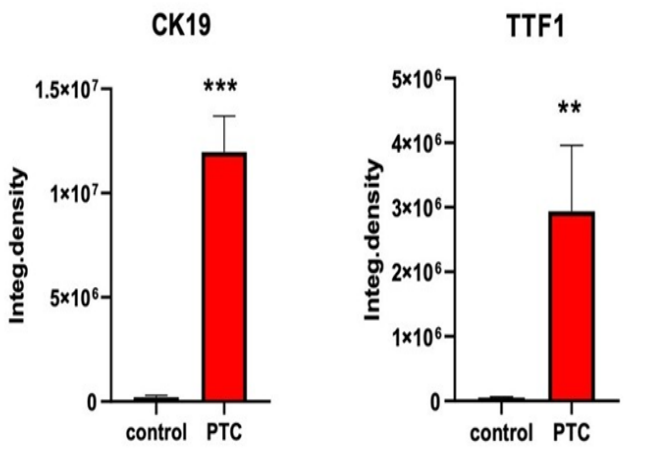
CK19
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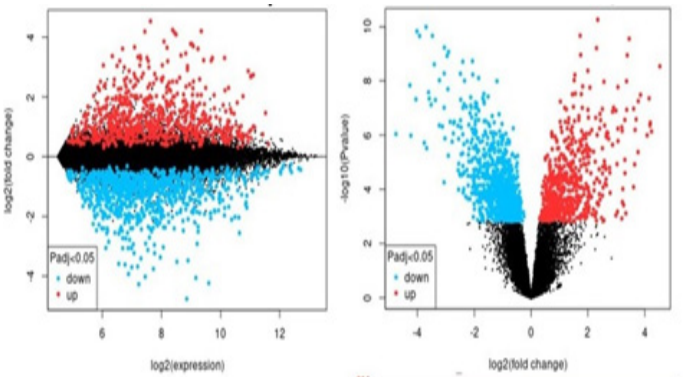
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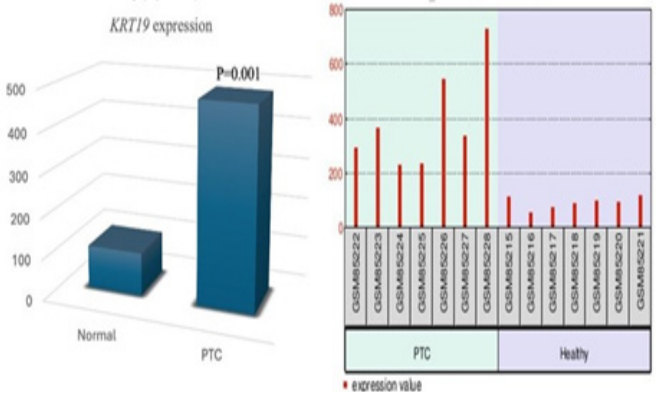
KRT19 expression
P=0.001
500
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300
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100
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Normal
PTC
PTC
Healthy
expression value