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# The expression of GAL-3 and CK-19 in Hashimoto's thyroiditis compared with papillary thyroid carcinoma

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**Objective** Investigate the hypothesis of that Hashimoto's thyroiditis (HT) consider risk factor to development papillary thyroid carcinoma (PTC) and to the compared expression of GAL-3 and CK-19 between groups of diseases that involve in this study.
**Methods** 27 paraffin-embedded tissue of HT submitted to examination by monoclonal antibody to CK-19 and GAL-3 by immunohistochemical test and compared with 24 cases of PTC, 7 PTC with HT and 23 nodular goiters as a control.
**Results** High positive expression of both markers in HT and there are non-significant differentiation between HT and PTC when $p > 0.05$.
**Conclusion** This study concludes that there is an etiological relationship between HT and development PTC and GAL-3 may have a role in the cellular transformation to a cancerous cell with PTC feature when continuous overexpression.
**Keywords** Hashimoto's thyroiditis, papillary thyroid carcinoma, cytokeratin-19, galectin-3

## Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disorder, which causes chronic lymphocyte inflammation due to a production of autoantibodies against thyroid antigens.[1] The prevalence of HT in female is more than male with a ratio of 8:1.[2] HT has a prevalence rate of 46 cases per 1000 individual.[2,3] It causes hypothyroidism,[4] and its characteristic by lymphocyte (T, B and plasma cell) infiltrate which makes follicles and germinal centers.[5] In HT, normal thyrocytes in some regions of thyroid transform into Hurthle cells or, and in other regions, destroy and atrophy.[6,7] There are many factors that play role in the pathogenesis of HT but the most important autoantibody–antigen complex fix by complement system causes complement-dependent antibody-mediated cytotoxicity.[8,9] increasing of T helper 17 lead to increase production IL-17 as pro-inflammatory molecules and defect or decrease T regulatory cell lead to decrease anti-inflammatory cytokines.[10] T helper 1 lymphocyte cell produce interferon gamma lead to recurrent and activation cytotoxic T lymphocyte (CD8+) which causes destruction thyroid follicular cell.[9,11]

Papillary thyroid carcinoma (PTC) is the widespread type of malignant thyroid tumor,[12,13] and is the seventh most prevalent cancer in women throughout the world.[14,15] PTC was predominant in female and the prevalence ratio in the female to male 2.5 to 4:1.[16]

The pathogenesis of PTC depending on the environmental, hormonal, and genetic factor may be effective to develop PTC.[17] One of mutation of RET/PTC,[18] BARF V600E,[19] and RAS[20] play a major role in the pathogenesis of PTC. Anyone of these mutations led to aberrant activation to the mitogen-activated protein kinase (MAPK) pathway,[21] and it is responsible for regulating cell growth, proliferation, differentiation, and mortality.[22]

Galectin-3 (GAL-3) is a carbohydrate-binding protein consisting of β-galactosides and domains of evolutionary carbohydrate-binding.[23] GAL-3 is responsible for coordinating the development (growth and differentiation) and apoptosis of cells, also it plays role in the migration of cells, may act as pathogens recognition receptors to recognize several structures on pathogens and regulate the production of some cytokine so that it has a role in innate immunity.[24] In many studies, it is used as a tumor marker for diagnosis and differentiation of PTC from benign thyroid lesion.[14,25–27]

Cytokeratin-19 is a protein which belongs to superfamily cytoskeleton proteins with a range of molecular weight from 40 to 68 kD, and are a largest and complexes group of intermediate filaments proteins.[28] So that transformed cells to the cancerous state from normal state characteristic by changes in the structure of cytoskeletal leading to increases of expression of CK-19 in the cancerous cell when compared with the normal cell.[29] Expression of CK19 was normally absent or focally in benign thyroid lesions while its expression in PTC was documented as strongly and diffusely.[30,31] CK-19 is used in many studies as a tumor marker for diagnosis and differentiation from benign thyroid lesion.[14,33,34]

This study was designed to investigate the hypothesis of that Hashimoto's thyroiditis consider risk factor to develop PTC and to the compared expression of GAL-3 and CK-19 between groups of diseases that are involved in this study.

## Materials and Methods

Eighty one retrospectively paraffin-embedded tissue samples with total or partial thyroidectomy included 24 cases of PTC, 7 PTC with HT, 27 Hashimoto's thyroiditis and 23 nodular goiters and were collected from November 2017 to March 2018 for 3 years (2015–2017) and the study design was a case-control study. The practical part of this study was conducted in the unit of histopathology of central laboratories of Imam AL-Hussein Medical City in Karbala. The gender of the selected sample included 11 males and 70 females and the ages were ranging from 20 to 72 years ago.

### *Samples criteria*

#### *Inclusion criteria*

(1) All cases related with age and gender, (2) cases from 1/1/2015 to 30/12/2017, (3) histopathological criteria of HT

was lymphoid follicle with germinal centers surrounded by lymphocyte infiltration, follicular destruction or atrophy, Hurthle cells presence sometimes. (4) Histopathological criteria of PTC enlarged nucleus and nuclear membrane irregularities resulting in loss of nuclear roundness, often presenting grooves in nucleus, architecture often (papillary, follicular, solid) and overlapping in the nucleus was moderate to severe.

*Exclusion criteria*

(1) Some of the blocks of sample was lost, (2) difficult take entire section from some blocks, (3) some archive slide of blocks of cases was lost, (4) element of inflammation in cases of nodular goiter as control, (5) PTC with element of inflammation except PTC with HT, (6) nodular goiters with hyperplastic, and (7) metastatic tumor to lymph nodes.

### Hematoxylin and eosin method

Two histopathologists read and exam archival hematoxylin and eosin staining slide to 81 samples to confirm the diagnosis of cases.

### Immunohistochemical method

Immunohistochemical staining was performed on tissue section with thickness 4 µm from blocks of archival paraffin-embedded tissue. All tissue section on the chargeable slide was deparaffinized three times with xylene and rehydrated with ethanol with three different concentrations (30%, 70% and 100%, respectively). The section of tissues subjected to Immuno DNA Retriever Citrate with PH 6 in container was put in a water bath for 60 min at 95–99°C. Immuno Detector Peroxidase Blocker (Bio SB, Glote, CA, USA) covered tissue section for 5 min then covered the tissue specimen with primary antibodies [anti-CK-19 and anti-GAL-3 (Bio SB, Glote, CA, USA)], The characteristics of primary antibodies were summarized in Table 1, after the diluted by Immuno Detector Protein Blocker/Antibody Diluent for 60 min followed by covered tissue section with Immuno Detector Biotin Link (Bio SB, Glote, CA, USA) for 10 min.

After biotin link step, the tissue section was covered with Immuno Detector HRP Label (Bio SB, Glote, CA, USA) for 10 min and to reveal the immune-staining, it was again covered with DAB substrate-chromogen solution (Bio SB, Glote, CA, USA) for 10 min, then to the final addition, tissue section was covered with hematoxylin as counterstain and finally slides were examined using an optical microscope (Olympus Dp72, Philippines). In this study, classical PTC was used as positive control for CK-19 and galectin-3 and thyroid tissue without primary antibody as negative control.

### Evaluation (reading and scoring system) immunostaining

According to the two histopathologists reading slides of IHC, the scoring system was divided into two groups: one was proportion of cell taken in immunostaining as in Table 2 and another one was intensity of immunostaining of taking cell as in Table 3.[35] The total score result was from multiplying the result of proportion (grade) with the result of intensity (intensity score) as in Table 4.[35–37]

### Statistical analyses

SPSS version 21 – software program was used to calculate all statistical process of result, chi-square and significances ($p > 0.05$) compared between groups using online web page www.socscistatistics.com.

## Results

The ages and genders of involved cases are illustrated in Table 5.

Table 1. **Illustrate the characteristics of primary antibodies**

| Reactivity | Dilution | Control | Localization | Isotype | Clone | Types | Antibodies |
|---|---|---|---|---|---|---|---|
| **Paraffin block** | 1:100 µL | Thyroid cancer | Cytoplasmic | IgG1/k | BSB-34 | Mouse monoclonal | Cytokeratin-19 |
| **Paraffin block** | 1:100 µL | PTC | Cytoplasmic | IgG1 | 9C4 | Mouse monoclonal | Galectin-3 |

Table 2. **Illustrate the system of the proportion of cells taken staining**

| Proportion (%) | Grades (G) |
|---|---|
| 0–10 | Grade 0 (Negative) |
| 11–30 | Grade 1 |
| 31–60 | Grade 2 |
| 61–90 | Grade 3 |
| 91–100 | Grade 4 |

Table 3. **Demonstrate the system of intensity of staining of cells**

| Observation | Intensity scores (IS) |
|---|---|
| Negative | Grade 0 (no staining) |
| Weak | Grade 1 |
| Moderate | Grade 2 |
| Strong | Grade 3 |

Table 4. **Illustrate total score**

| Meaning | Interpretation | Total score(G * IS) |
|---|---|---|
| Negative | Negative | 0 |
| Weak positive | +1 | 1–4 |
| Moderate positive | + 2 | 5–8 |
| Strong positive | +3 | 9–12 |

Table 5. **Frequent and proportion of cases with ages group and genders**

| Cases (No.) | Age groups (%) | | | Genders (%) | |
|---|---|---|---|---|---|
| | <40 | 40–59 | ≥60 | Females | Males |
| HT (27) | 10 (37.03) | 12 (44.44) | 5 (18.51) | 23 (85.19) | 4 (12.9) |
| PTC (31) | 19 (61.29) | 10 (32.25) | 2 (6.45) | 27 (87.1) | 4 (14.81) |

HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma.

### *Expression of CK19 and galectin-3 in PTC and normal thyroid tissues in nodular goiter*

Expression of CK19 in PTC 29/31 (93.5%) with mostly strong positively (+3), while CK19 expression in N.G 14/23 (60.9%) with mostly weak positively (+1) (Table 6).

About 26 samples of PTC was submitted to expression of GAL-3 in PTC 20/26 (76.9%) with mostly weak positively (+1) while GAL-3 expression in N.G 3/23 (13%) with mostly weak positively (+1) but the negativity of GAL-3 in N.G 20/23 (87%) (Table 6). There is a significant comparison by CK-19 and GAL-3 expression between PTC and N.G (Table 7).

### *Expression of CK19 and Galectin-3 in HT*

About 27 samples of HT was submitted to examination by CK-19, 26/27 (96.3%) mostly strong positively (+3) while 26 sample submitted to examination by GAL-3, 24/26 (92.3%) with mostly equally moderate and weak positively expression (equally +2, +1) (Table 6).

There are non-significant analysis or comparison by CK-19 and GAL-3 expression between PTC and HT but there are significant analysis or comparison by CK-19 and GAL-3 expression between HT and N.G (Table 7). This means, the specific diagnostic markers for PTC was expressed in HT.

## Discussion

Inflammation diseases as autoimmune diseases or infection by virus or bacteria or parasite of the specific organ could be considered as a predisposing factor or risk factor to the development of cancer to the same organ such as autoimmune pancreatitis linked with high risk of development of pancreatic cancer,[38] pelvic inflammatory disease increase risk of development of ovarian carcinoma,[39] and development of colorectal carcinoma elevated in patients with ulcerative colitis.[40,41]

In this study, we used two immunohistochemical markers to examine the presence of etiological relationship between HT and development of PTC, 96.3% of cases of HT expression of CK-19 and 92.3% of HT expression of GAL-3, this result semi-symmetric to result of expression of these markers in PTC and there are non-significant differentiation between HT and PTC, so that our result directed to confirm HT consider risk factor for development PTC.

Our study agreement of Ma et al.'s[42] result, when they found CK-19 expression in FED was lower than in PTC while significantly higher than control and no differences between normal follicular cell and cell of HT but there are differences between PTC and normal follicular cell by expression of GAL-3 but they illustrate expression of GAL-3 in HT was abnormal and may be having relationship with neoplastic change. As well, our study agree to the result of Chui et al. They found immunohistochemical profiles expression in FED of chronic lymphocyte thyroiditis identical to the expression of profiles in PTC and cannot differentiate between FED and PTC in the thyroid with chronic lymphocyte thyroiditis by CK-19 and GAL-3 expression.[43]

And there are some previous studies found wrong in interpretation of immunomarker, Nasr et al.[44] examination morphologic features, immunohistochemical staining, and molecular testing to 59 cases of HT, they found 12 cases only contain HBME1+ and CK19+ atypical cell clusters and this the 12 cases examined for BRAF mutation and the result of molecular study was negative for this mutation so that Nasr et al. suggested the atypical cell clusters in HT may not be preneoplastic and should be exercised in interpretation of immunohistochemical staining. Other study found not differs statistically between HT and control. In a study conducted to detect diagnostic value of immunohistochemical profiles (CK-19, GAL-3 and others) in PTC, when $p < 0.05$ consider significant analysis, they found significant analysis in PTC when compared with N.G and HT ($p < 0.001$) but when compared HT with N.G there are no significant analysis ($p > 0.72$ for CK-19 and $p > 0.785$ for GAL-3) when they exam 120 cases PTC, 34 cases N.G, and 28 cases HT.[45] Our result differ completely from the result of Huang et al. because there are significant analysis between HT and N.G and non-significant analysis between PTC and HT.

But there are several studies prove the hypothesis HT risk factor to development of PTC by methods rather immunohistochemical method such as Kang et al.[46] found molecular link between oxyphil cell metaplasia in HT and development of PTC when they exam RET/PTC rearrangement, RAS and BRAF mutation in HT, PTC and normal of thyroid tissue and Azizi et al.[47] examine correlation HT and thyroid cancer in patients with thyroid nodules by FNA biopsy and serology measurement of anti-TPO antibody and anti-TG antibody, the association of HT with thyroid cancer is antibody specific.

Some previous studies show that GAL-3 have role in cellular transformation to PTC,[48,49] and overexpression of GAL-3 in mouse with mutant K-RAS with Pancreatic carcinoma but when decreased expression of GAL-3 they demonstrate volume of cancer, cell proliferation was reduced in mouse with mutant K-RAS with pancreatic cancer[50] so that GAL-3 may have a role in development PTC.

Table 7. **Illustrate significant and non-significant analysis between group of studies**

| CK-19 expression | | |
|---|---|---|
| PTC vs. N.G | PTC vs. HT | HT vs. N.G |
| Sig. $p < 0.05$ | Non-sig. $p < 0.05$ | Sig. $p > 0.05$ |
| **GAL-3 expression** | | |
| Sig. $p < 0.05$ | Non-sig. $p < 0.05$ | Sig. $p < 0.05$ |

HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma; N.G, nodular goiter; CK-19, cytokeratin-19; GAL-3, galectin-3.

Table 6. **Illustrate the score of staining cells of marker galectin-3 and cytokeratin-19**

| Markers | PTC | | | | No. | HT | | | | No. | N.G | | | | No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | +1 | +2 | +3 | | N | +1 | +2 | +3 | | N | +1 | +2 | +3 | |
| CK-19 | 2 | 6 | 4 | 19 | 31 | 1 | 6 | 9 | 11 | 27 | 9 | 10 | 2 | 2 | 23 |
| GAL-3 | 6 | 10 | 7 | 3 | 26 | 2 | 9 | 9 | 6 | 26 | 20 | 2 | 1 | 0 | 23 |

HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma, N.G, nodular goiter; CK-19, cytokeratin-19; GAL-3, galectin-3.

## Conclusion

When we used the specific marker of immunohistochemical test for diagnosis of PTC and show these markers express in HT so that we conclude that there is an etiological relationship between HT and development PTC and also we conclude that GAL-3 may have a role in the cellular transformation to a cancerous cell with PTC feature when continuous overexpression.

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## Conflict of Interest

None.

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