Immunohistochemical expression of ALHD1A1 in thyroid goiter and normal peritumor tissue around papillary thyroid carcinoma in a sample of Iraq patients

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ABSTRACT

Background: Papillary thyroid carcinoma (PTC) is the most common type of thyroid malignancy and has one of the most increasing incidence among other cancers in the United States. According to the theory of cancer stem cells (CSCs), it has been established that expression of aldehyde dehydrogenase (ALDH1A1) has been linked to play a fundamental role in PTC initiation, proliferation, invasiveness, local recurrence metastasis and chemotherapeutic resistance. However, ALDH1A1 expression patterns in the normal tissue around PTC (NPT) and comparing it with that of multinodular goiter (MNG) have not been well established.

Materials and methods: A total of 70 patients included in this study, mostly admitted to Al-Yarmouk teaching hospital/Baghdad. 50 cases of PTC including safety margins with 5 cases positive nodal metastasis and 20 cases of MNG tissue specimens have been prepared to be stained immunohistochemically with anti-ALDH1A1, and compared the expression profiles between the two groups and with age and gender.

Results: Overexpression of ALDH1A1 was significant in the NPT group compared to that of MNG in regards to the percentage of immunopositive cells and staining intensity. NPT with nodal metastasis group showed even higher significant difference than metastasis negative NPT group (P-value<0.01).

Conclusion: ALDH1A1 expression in normal tissue around PTC was similar to that of PTC and higher than MNG tissue.

Keywords: immunohistochemistry, papillary thyroid carcinoma, ALDH1A1, multinodular goiter

INTRODUCTION

Thyroid cancer is the most prevalent malignant tumor of endocrine glands and the first cause of death among endocrine cancers, it is the fifth most common cancer in women in the US and the eighth most diagnosed malignancy in Iraqi women (1)(2).Although it mainly affects women, it can also be diagnosed in men with male to female ratio of 1:3(3).Recently, epidemiological studies have shown that the incidence of thyroid cancer is rising, more than any other malignant tumor(4).In the United States, the incidence of thyroid cancer over the past 25 years has been reported to be 4 times (from 8000 to 54000 patients)(5). Differentiated thyroid cancers are most common type of thyroid malignancy, of which papillary thyroid carcinoma (PTC) represents the most common thyroid cancer accounting for 80-85% of thyroid tumors(6).Despite the fact that PTC has good prognosis (7),However, about 10–20% of stage I/II papillary thyroid carcinoma patients have disease relapse (recurrence), suffering invasive tumors and/or distant metastases(8)(9).There have been many risk factors for PTC including multinodular goiter (MNG), MNG is the most common thyroid disorder affecting approximately 7% of world’s population (10).Risk evaluation of malignant transformation of MNG to PTC has been studied extensively. Surgical specimens of nodular goiters have shown that thyroid cancer was found in 4-17% (11). It has been shown that patients with multinodular goiter and benign fine needle aspiration (FNA) have demonstrated a 46.3% significant incidence of thyroid cancer, often of papillary carcinoma variety (12).
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It is well recognized that cancer stem cells (CSCs) theory is firmly established to explain thyroid cancer cells heterogeneity and tumorigenesis and addressing some critical aspects tumor progression, local disease recurrence, metastasis as well as resistance to chemo-/radiotherapy (13). CSCs are sharing many molecular resemblances with both embryonic and adult stem cells, and their identification is possible by expression of stemness biomarkers and the ability to create spheres of cells (called thyrospheres) in vitro and having the ability to induce tumor formation when injected into immunodeficient mice (in vivo) (14). Aldehyde dehydrogenase (ALDH) is a member of the ALDH superfamily, which comprised of 19 isozymes (15). ALDH1 is the main variant of this major family and ALDH1A1 has been reported as an important CSC marker in PTC (16). ALDH1 has a major role in controlling an important pathways promoting CSC tumorigenesis and stem cell signaling. ALDH enzymes are controlled by retinoic acid derivatives including retinoid chemotherapeutic agents, and perhaps some oncogenic pathways (e.g. WNT/beta-catenin and MUC1-C/ERK). Therefore, ALDH enzymes actively contribute in oxidizing aldehydes that induce cellular or genetic damage or participating in signaling processes, furthermore, reducing ROS production and effects on cellular quality (17). ALDH1A1 involved in cytosolic production of retinoic acid via oxidation of toxic aldehydes to less toxic carboxylic acid and retinol to retinoic acid, allowing the cell to detoxify from chemotherapeutic drugs and reactive oxygen species (ROS) and also preventing genetic damage by oxidative stress of ROS (18). Retinoic acid is critical for regulation of gene expression in CSC promoting stemness, differentiation, mediating tumorigenesis and maintaining CSC survival (17)(19).

AIM OF THE STUDY

Our current study aimed to evaluate the difference of immunohistochemical expression of ALDH1A1 between MNG tissue and normal peritumor tissue of PTC.

MATERIALS AND METHODS

Patients’ data

This study was conducted over a period of one year from April 2018 to March 2019 on patients admitted to Al-Yarmouk teaching hospital in Baghdad and few cases has been collected from private laboratories. Current study was performed on tissue specimens from total number of 70 patients (55 females and 15 males) who had been admitted to surgery department and undergone thyroidectomy surgery for treatment of goiter. As confirmed by reviewing a newly prepared hematoxylin and eosin (H&E) stained slides, 50 patients have had a diagnosis of papillary thyroid carcinoma, of which, 5 cases reported positive nodal metastasis, while the remaining 20 had multinodular goiter. Patient age, gender and metastatic state have been obtained. Ethical approval for this research has been granted from the ethics committee of Al-Yarmouk teaching hospital affirmed by written consent obtained from all patients. All patients were euthyroid on clinical picture and biochemical assessment (serum thyroid stimulating hormone TSH and thyroxine T4) and none have received any thyroid-related treatment. Patients with previous history of chemotherapy or radioactive iodine therapy have been excluded.

Specimens’ collection

Tissue specimens were divided into two groups; namely normal peritumor group (NPT) includes 50 patients diagnosed with papillary thyroid carcinoma, tissue specimens surgically removed with safety border, with mean age of 36.94 years (±8.2 years), and age ranged 26-55 years. Other group called multinodular goiter (MNG) includes 20 patients having diagnosed with benign multinodular goiter, mean age 34.68 (±7.7 years) with a range extends 25-50 years.

Immunohistochemistry

All tissue specimens were processed to be embedded in paraffin blocks according to a standard protocol (formalin-fixed, paraffin embedded)(20). For each case 2 serial sections were obtained, with 4 µm thickness. One section was stained with H&E to review and confirm the diagnosis. The other section was placed on a positively charged slides and dewaxed with xylene, then gradually hydrated. Citrate buffer solution was used for antigen retrieval where the slides are placed in hot bathed containers for 20 minutes. Followed by peroxidase block when slides were incubated in humidified chambers and then protein block was used before treating the slides with primary anti-ALDH1A1 antibody (primary antibody; Abnova®, Taiwan, clone 152CT1.2.2 Catalog no. MAB12300, mouse anti-human monoclonal antibody), which was diluted as 1:100 using antibody diluent solution (Abcam® USA, code ab64261). The secondary detection kit (Abcam® USA, code ab80436) mouse specific HRP/DAB was used based on labelled streptavidin-biotin technique. Followed by DAB and then stained with chromogen. Then all slides were counterstained with hematoxylin and mounted using DPX (21).
Assessment of Immunohistochemical expression

All tissue slides were assessed using light microscope without earlier knowledge of the patients group or the age of the patient. Anti-ALDH1A1 expression depicted a distinct cytoplasmic brown staining. The slides have been examined with low magnification power 10X to identify areas with high immunostaining, if no staining was shown at low power repeating the examination with high magnification power 40X to identify areas of weak immunostaining. In each slide, 5 fields were assessed and examined for ALDH1A1 immunoreactivity. Expression of immunoreactivity of ALDH1A1 was evaluated semi-quantitively as previously reported (22). Marker immunostaining was scored using extent of staining (proportion or percentage of stained cells) and staining intensity. Percentage of staining was scored as follows; 0 = < 5%, 1= 5-20% of cells stained positively, 2= 20-50%, 3= 50-80%, 4= > 80%. However, intensity of immunoreactivity was scaled as following: 0: negative, 1+: weak, 2+: moderate and 3+: strong. Total score of ALDH1A1 immunoreactivity was calculated by multiplying the percentage of stained cells and the intensity score (scores ranged 0-3) (23).

Quality control

Normal colon tissue sample from a control colonoscopy was included for ALDH1A1 and considered as a positive control (24). While negative control was prepared by deleting the primary anti-ALDH1A1 antibody and adding antibody diluent only and continue with the same steps in immunohistochemistry.

Statistical analysis

Data analysis was performed using SPSS (Statistical Packages for Social Sciences, IBM® USA, version 24). Qualitative variables were analyzed by means of percentage, mean and range (minimal-maximal values). Qualitative variables were statistically analyzed using Pearson Chi-square test and independent sample student t-test.

RESULTS

Patients’ age and gender

The mean age of NPT group was 36.94 (±8.2) years with a range extends (26-55) years. The mean age of MNG group 34.68 (±7.7) years with a range extends (25-50) years. There was no significant difference between age means of both NPT and MNG groups (P-value = 0.2) (Figure 1). There was a trend of increasing total ALDH1A1 expression score with increasing age (R-value = 0.064), however, statistically non-significant (P-value > 0.05)

![Figure 1 Age distribution among normal peritumor tissue and goiter groups (Bars represent mean, error bars=standard deviation).](http://doi.org/10.36295/ASRO.2020.231347)
Male patients in NPT group showed significantly higher total ALDH1A1 scores (2.39±0.43) compared to female patients total score (1.56±0.55) (P-value = 0.039). While in multinodular goiter female patients showed higher score compared to male patients (P-value<0.05) (Figure 3).

**Immunohistochemical expression of ALDH1A1**

**Immunostaining percentage of ALDH1A1**


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The mean of expression percentages of ALDH1A1 in NPT group was 78% (±9.5%), comparing to MNG group mean expression percentage 73.7% (±11.3%), the difference was not statistically significant between the two groups (P-value>0.05) (Figure 4).

**Immunostaining intensity of ALDH1A1**

Neither NPT nor MNG groups depicted negative (0) staining intensity or weak (1+) intensity. However, NPT group displayed significantly higher percentage of cells showing strong (3+) staining intensity (63%) compared to MNG group (13%) (P-value<0.01). While MNG group demonstrated higher moderate intensity (2+) (P-value<0.01) (Figure 5).

**Immunostaining total score of ALDH1A1**

Total score data were represented and statistically analyzed as mean (±SD). In NPT group of cases, the mean of ALDH1A1 expression score was 2.1 (±0.56). Contrariwise, the mean of ALDH1A1 scoring in MNG group was 1.55 (±0.22). Comparing the means of two groups using independent sample’s student’s t-test, the mean of ALDH1A1 scores in NPT group was statistically significant higher than mean scores in MNG group (P-value<0.01) (Figure 6).

![Expression Percentage (%) of ALDH1A1](image_url)

*Figure 4 ALDH1A1 expression Percentage (%) in different thyroid disorders. (Bars represent mean, errors bars=standard deviation).*
**Figure 5** ALDH1A1 expression intensity in different thyroid disorders. (Bars represent mean, errors bars=standard deviation, **=P-value<0.01).

**Figure 6** ALDH1A1 expression total score in different thyroid disorders. (Bars represent mean, errors bars=standard deviation, **=P-value<0.01).
Figure 7 Microphotograph depicts immunohistochemical expression of ALDH1A1 in NPT tissue shows 3+ cytoplasmic/nuclear immunoreactivity for ALDH1A1 antibody. X400.
Figure 8: Microphotograph depicts immunohistochemical expression of ALDH1A1 in MNG tissue shows 2+ cytoplasmic/nuclear immunoreactivity for ALDH1A1 antibody. X400.
Immunostaining total score of ALDH1A1 in relation to metastasis
NPT group with positive nodal metastasis exhibited significantly higher ALDH1A1 expression total score (2.75±0.13) compared to negative metastasis NPT group (1.95±0.49) (P-value<0.01) as shown in Figure 10.
ALDH1A1 has been regarded as a significant tumor biomarker in many cancer types (25). The function of ALDH1A1 enzyme is crucial for stem cell differentiation and maintaining its survival (26). Injecting immunocompromised (non-obese diabetic/severe combined immune deficient NOD/SCID) mice subcutaneously with PTC cells (as few as 5000 cells) that have high ALDH1 expression resulted in generation of tumors (thyrospheres) having the same phenotype of its origin (i.e. PTC) in the injected mice implying tumorigenic characteristic of these cells, comparing to injecting 25000 cells with negative ALDH1 that did not develop a tumor. Additionally, injecting 100 cells derived from thyrospheres resulted in augmenting lung metastasis but not the thyroid tumor (27). Furthermore, ALDH1A1 has been shown to have an important role in predicting the aggressiveness of PTC and hence having a potential prognostic value for the diagnosis of PTC (22).

In this study, we found that about 78% positive cells of NPT group of patients with intensity ranging from 2+ to 3+ and total score of 2.1, compared to MNG group with positivity of 73.7% with expression intensity also ranges from 2+ to 3+ and total expression score of about 1.55. Although both NPT and MNG groups show ALDH1A1 positivity that seems to be equal however, NPT group exhibited highly significant expression intensity than MNG resulting in higher total score of ALDH1A1 immunostaining. These results are consistent with findings from many researchers (27)(28)(22). However, one study revealed that PTC cells showed ALDH1 more than ATC and benign thyroid disorder (29). Nevertheless, other studies have described higher rates for ALDH1A1 expression positivity in normal thyroid tissue as well as PTC (30)(31)(32)(33). Although the possible explanation for these discrepancies is not well established yet (14), besides, the current evidence is strong enough to support the clinical significance of ALDH as biomarker for CSC in thyroid cancer, one study suggested that ALDH should not be utilized as a marker for CSC in tissues that show high ALDH expression under normal conditions (34)(31).

It is well recognized that ALDH enzymes and overall activity have been involved in resistance to radiotherapy and chemotherapy, as well as tumor recurrence and aggressiveness in several solid tumors including thyroid, breast and esophagus (35)(36)(37). This could suggest that understanding the biological patterns of ALDH1A1 expression in PTC and other related tissues could provide a better insights into explaining and predicting of drug resistance in thyroid malignancy. Moreover, ALDH could be implicated as a prognostic biomarkers for cancer aggressiveness, disease recurrence, metastasis and overall survival (38). One study has reported that anaplastic thyroid carcinoma related CSCs have demonstrated overexpression of ALDH compared to less aggressive
papillary thyroid cancer, and this expression was correlated with poor patient survival as well as co-expression of chemotherapy resistance-related proteins (29).

In this study, we observed an increased ALDH1A1 expression score with increasing age (although statistically non-significant), with slight male predominance especially in NPT group. This finding coincides with many authors (34)(22)(28). However, a recent study by Kim and Koo has shown no correlation between different gender and age with ALDH1A1 expression in different thyroid tissues (39).

Despite the fact that ALDH1A1 appears to be a beneficial prognostic marker for tumor aggressiveness, drug resistance and patient overall survival (17), few studies attempted to target ALDH as a therapeutic goal for the purpose of eliminating CSC and preventing disease recurrence. These attempts remain controversial for many reasons, there are 19 isoforms of ALDH enzymes throughout the body. Additionally, there is some evidence that suggests a significant overlap between those ALDHs and thus targeting one could affect others or result in compensating from others (40).

CONCLUSION

In our current study, we concluded that normal peritumor tissue around PTC demonstrated ALDH1A1 overexpression which may resemble that of the cancer tissue itself, implying a potential predictive significance for disease recurrence and chemotherapy resistance after surgery within remaining normal tissue. CSC markers may be considered as a diagnostic tool for risk assessment of malignant transformation to PTC.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


