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Expression of CK19, CD56, and Galectin-3 Tumor Markers in Different Types of Thyroid Carcinoma

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ABSTRACT

Background: *Thyroid disorders represent a major problem in Saudi Arabia, particularly in the northern part of the country. The objective of the current study was to investigate the immunohistochemical expression of CK19, CD56, and Gal-3 in a series of Saudi patients with thyroid carcinoma in Northern Saudi Arabia (Hail) region.* ***Methodology:*** *This is a retrospective study, investigated 50 formalin-fixed paraffin wax tissue blocks with a confirmed diagnosis of thyroid carcinoma from Northern Saudi Arabia. Immunohistochemistry demonstration was for CK19, CD56, and Gal-3 markers applying Avidin-Biotin method.* ***Results:*** *Out of the 50 patients, 45(90%) were females and 5(10%) were males, aged 13-70 years old with a mean age of 43 years. CK19 was positively expressed in 74% of the thyroid carcinoma. Positive CD56 expression was demonstrated in 45.7%, 58.3%, and 100% of the papillary, follicular and undifferentiated thyroid carcinomas, correspondingly. Positive Galectin-3 expression was demonstrated in 71.4%, 58.3%, and 100% of the papillary, follicular and undifferentiated thyroid carcinomas, correspondingly.* ***Conclusion:*** *Papillary thyroid carcinoma is the most common thyroid cancer in Northern Saudi Arabia. Females represent more than 90% of the cases of the thyroid carcinoma in Northern Saudi Arabia. CK19, CD56, and Gal-3 are useful for the assessment of thyroid carcinoma.*

Keywords: Thyroid cancer, Papillary carcinoma, Follicular carcinoma, Saudi Arabia, CK19, CD56, Gal-3

INTRODUCTION

In recent decades, the incidence of thyroid cancer has been noticeably increasing worldwide, with declined male mortality in numerous countries [1]. Several factors have been involved in the apparent increased incidence of thyroid cancer including the application of more sensitive diagnostic, increased population exposure to radiation, increased of true cases, increase iodine intake, chronic lymphocytic thyroiditis, environmental pollutants, and the possible unrecognized carcinogens [2-4]. The declines in the mortality of thyroid cancer might be due to variations in risk factor exposure and changes in the diagnosis and treatment of the disease [1].

In Saudi Arabia, the incidences rates of thyroid cancer are increasing during the few past years with significant geographical variation throughout the country. Thyroid cancer was previously reported as the second most frequent female's cancer with a females' males' ration at 1.00 to 0.30. The increased incidence might be due to increased detection with the application of advanced diagnostic technology, increased of true cases, and exposure of determinant environmental pollutants in different regions [5,6].

Cytokeratin 19 (CK19) is a useful immunohistochemical marker, which can be used successfully in thyroid tumors to recognize thyroid cancer, particularly papillary carcinoma [7]. CD56 (cell adhesion molecule) is expressed by several immune cells (particularly T-cells). Both numerical and functional insufficiencies and phenotypic changes of the CD56+ immune cell fraction have been described in patients with immune-related disorders and malignant conditions [8]. The combined use of CK19 and CD56 is useful in discerning papillary thyroid carcinoma and its variants from

other imitating thyroid tumors [9]. Galectin-3 (β galactoside-binding protein) (Gal-3) is an essential regulator of a wide variety of malignant cell events and plays vital parts in malignant cell growth, transformation, apoptosis, angiogenesis, adhesion, invasion, and metastasis [10]. Gal-3, which has established important consideration for its efficacy as a diagnostic marker for thyroid carcinoma, embodies a well-investigated molecular contestant for thyroid cancer diagnosis [11]. Therefore, the present study aimed to investigate the immunohistochemical expression of CK19, CD56, and Gal-3 in a series of Saudi patients with thyroid carcinoma in Northern Saudi Arabia (Hail) region.

MATERIALS AND METHODS

In this retrospective study, 50 formalin-fixed paraffin wax tissue blocks were retrieved with their related records from the Department of Histopathology at the King Khaled Hospital (Hail, Saudi Arabia) during the period from September 2017 to August 2018. Tissue samples were re-sectioned and the tissue sections were used for confirming a thyroid cancer diagnosis, performing of CK19, CD56, and Gal-3 immunohistochemical testing.

The immunohistochemistry demonstration was for CK19, CD56, and Gal-3 markers applying Avidin-Biotin method described elsewhere [12]. Obtained sections were initially de-waxed on a hot plate oven and cleared in 2 changes of xylene 2 minutes in each, hydrated through descending grades of ethanol (100%, 90%, 70%, 50%, and water) 2 minutes in each. The sections were boiled in the Target Retrieval Solution of Dako (citrate buffer solution pH 6) in a water bath at 95°C for 30 minutes, then left to cool at room temperature and washed 3 times with PBS. The 3% hydrogen peroxide in methanol was added to each section for 15 minutes to block endogenous peroxidase activity, and then washed 3 times with Phosphate Buffer Saline (PBS) pH 7.4 for 3 minutes, then the diluted monoclonal mouse anti-human (for each marker) from Dako company was added to each slide for 30 minutes, washed in Phosphate Buffer Saline (PBS) for 3 minutes, then treated with biotinylated link for 15 minutes, washed in PBS for 3 minutes, treated in conjugated streptavidin, washed in PBS for 3 minutes, then treated with 3,3-diamino-benzidine-tetrahydrochloride (DAB) for 10 minutes, washed in PBS for 3 minutes, then stained in Mayer's Hematoxylin as counterstain for one minute, then washed and blued in running tap water, hydrated, cleared and mounted in DPX. Positive and negative controls from known positive and negative samples were treated the same as the samples.

Ethical Consent

Our study protocol was confirmed according to the 2013 Declaration of Helsinki and this study was approved by the ethics committee of the College of Medicine, University of Hail, Saudi Arabia.

Statistical Analysis

Data analysis was done by using the Statistical Package for Social Sciences (SPSS version 16; SPSS Inc, Chicago, IL). SPSS was used for analysis and to do Fisher exact test for statistical significance ($p<0.05$ was considered significant). The 95% confidence level and confidence intervals were employed.

RESULTS

This study evaluated the immune-expression of CK19, CD56, and Gal-3 in different types of thyroid carcinoma in 50 Saudi patients with thyroid carcinomas. Out of the 50 patients, 45 (90%) were females and 5 (10%) were males, aged 13-70 years old with a mean age of 43 years. Most of the patients were at the age group ≥ 51 years followed by the age ranges ≤ 30 years and 41-50 years, representing 15/50 (30%), 12/50 (24%) and 10/50 (20%), respectively. Most of the patients were diagnosed with papillary thyroid carcinoma followed by follicular thyroid carcinoma and undifferentiated thyroid carcinoma, representing 35/50 (70%), 12/50 (24%), and 3/50 (6%), as indicated in Table 1 and Figure 1.

Table 1 Distribution of the patients by sex, age and thyroid carcinoma type

Variable	Females	Males	Total
Age (Years)			
≤ 30	13	0	13
31-40	10	2	12
41-50	9	1	10
51+	13	2	15
Total	45	5	50
Thyroid Carcinoma type			
Papillary carcinoma	31	4	35

Follicular carcinoma	12	0	12
Undifferentiated carcinoma	2	1	3
Total	45	5	50

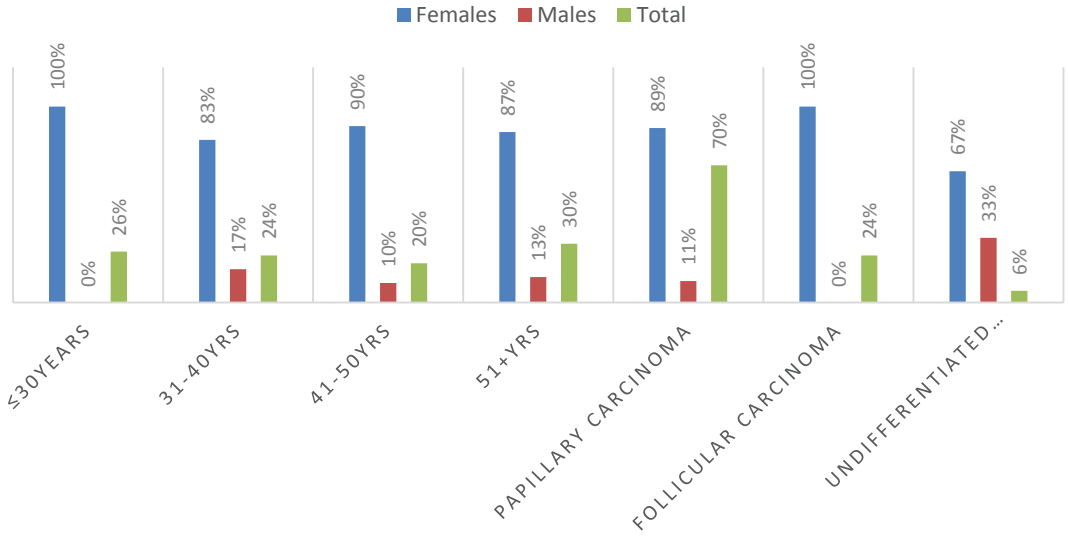

Figure 1 Description of patients by sex, age and thyroid carcinoma type

The majority of papillary thyroid carcinoma cases were associated with the age range ≤ 30 years, followed by age groups 31-40, and 41-50 years, constituting 11/35 (31.4%), 10/35 (28.6%), and 8/35 (22.9%), in this order. Most follicular carcinoma cases were found at age group ≥ 51 years 6/12 (50%). All cases of undifferentiated thyroid carcinoma were detected in the age group ≥ 51 years 3/3 (100%), as shown in Table 2 and Figure 2.

Table 2 Distribution of the patients by age and thyroid carcinoma type

Thyroid Carcinoma type	≤ 30 years	31-40 yrs	41-50 yrs	51+ yrs	Total
Papillary carcinoma	11	10	8	6	35
Follicular carcinoma	2	2	2	6	12
Undifferentiated carcinoma	0	0	0	3	3
Total	13	12	10	15	50

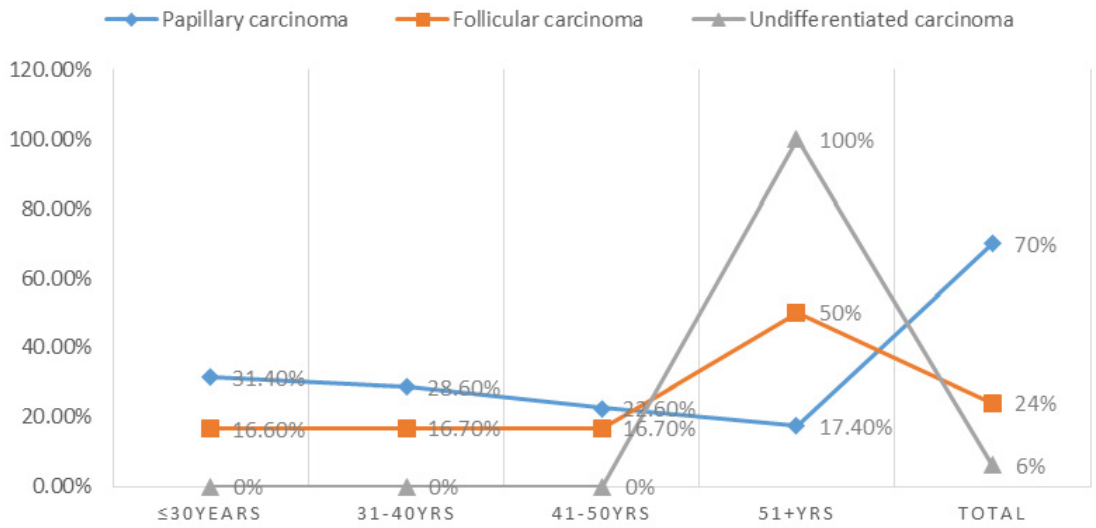

Figure 2 Description of the patients by age and thyroid carcinoma type

Table 3 and Figure 3 summarizes the distribution of the patients by tumor markers and thyroid carcinoma type. CK19 was positively expressed in 37/50 (74%) of the thyroid carcinomas. Out of the 35 cases of papillary carcinoma, 26/35 (74%) were positive and the remaining 9/35 (26%) were negative. Follicular and undifferentiated carcinoma cases expressing positive CK19 were 8/12 (66.7%) and 3/3 (100%), respectively. Positive CD56 expression was demonstrated in 16/35 (45.7%), 7/12 (58.3%), and 3/3 (100%) of the papillary, follicular and undifferentiated thyroid carcinomas, correspondingly. Positive Galectin-3 expression was demonstrated in 25/35 (71.4%), 7/12 (58.3%), and 3/3 (100%) of the papillary, follicular and undifferentiated thyroid carcinomas, correspondingly.

Table 3 Distribution of the patients by tumor markers and thyroid carcinoma type

Tumor Marker	Papillary Carcinoma	Follicular Carcinoma	Undifferentiated Carcinoma	Total
CK19				
Positive	26	8	3	37
Negative	9	4	0	13
Total	35	12	3	50
CD56				
Positive	16	7	3	26
Negative	19	5	0	24
Total	35	12	3	50
Galectin-3				
Positive	25	7	3	35
Negative	10	5	0	15
Total	35	12	3	50

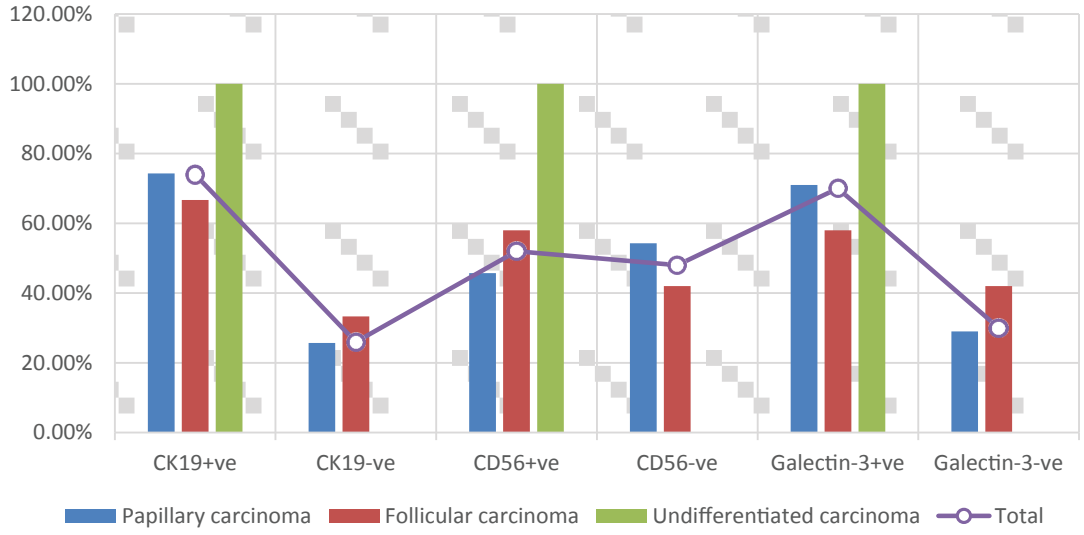

Figure 3 Description of patients by tumor markers and thyroid carcinoma type

Table 4 summarizes the distribution of the patients by the tumor markers expression and age. CK19 was predominantly positively expressed in the age groups ≤ 30 years and 51+ years representing 11/37 (30%) in each. The majority of CD56 positive expressions were noted in the age group 51+ years 12/26 (46%). High Galectin-3 positive expression was observed in ≤ 30 years demonstrating 11/35 (31%) (Figures 4-7).

Table 4 Distribution of the patients by age and tumor markers expression

Thyroid Carcinoma type	≤ 30 years	31-40 yrs	41-50 yrs	51+ yrs	Total
CK19					
Positive	11	5	10	11	37
Negative	2	7	0	4	13
Total	13	12	10	15	50

CD56					
Positive	4	6	4	12	26
Negative	9	6	6	3	24
Total	13	12	10	15	50
Galectin-3					
Positive	11	5	9	10	35
Negative	2	7	1	5	15
Total	13	12	10	15	50

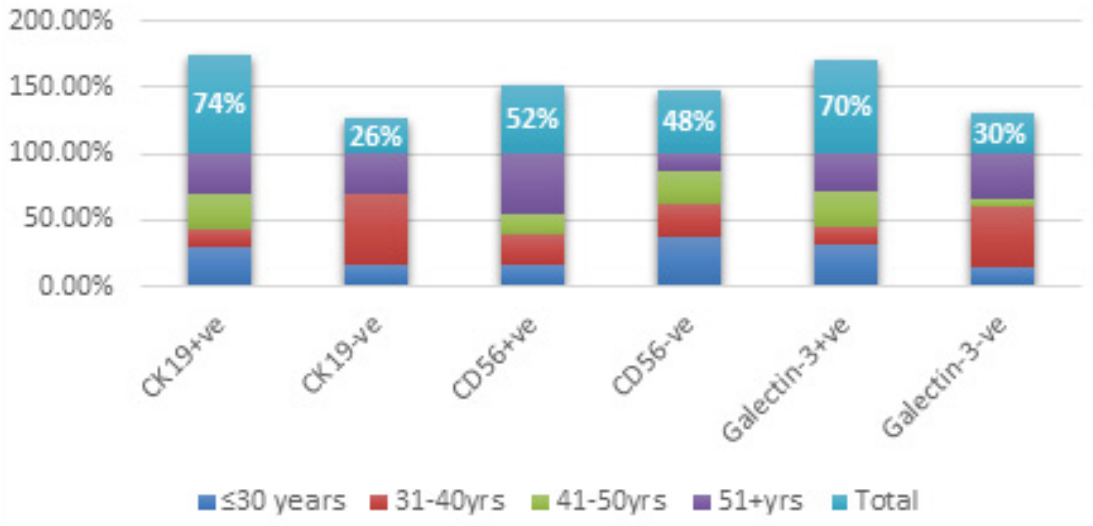

Figure 4 Description of the patients by age and tumor markers expression

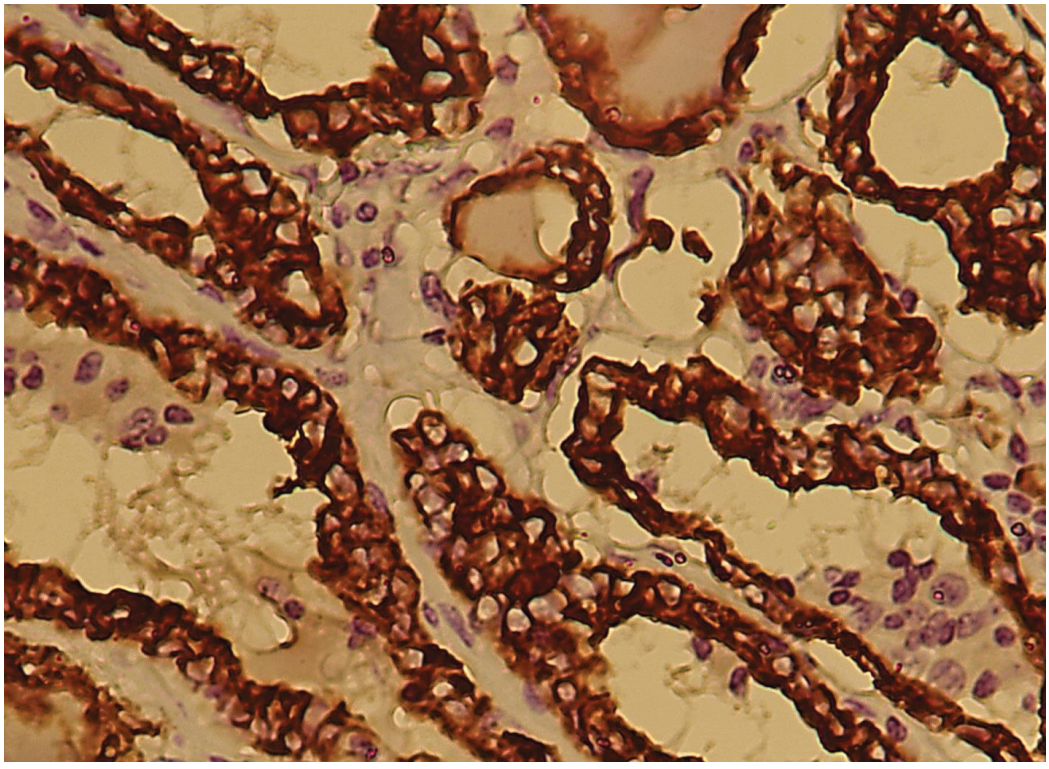

Figure 5 Ck19 immunohistochemical staining of thyroid carcinoma applying Avidin-Biotin method. Brown DAB staining indicating positive expression

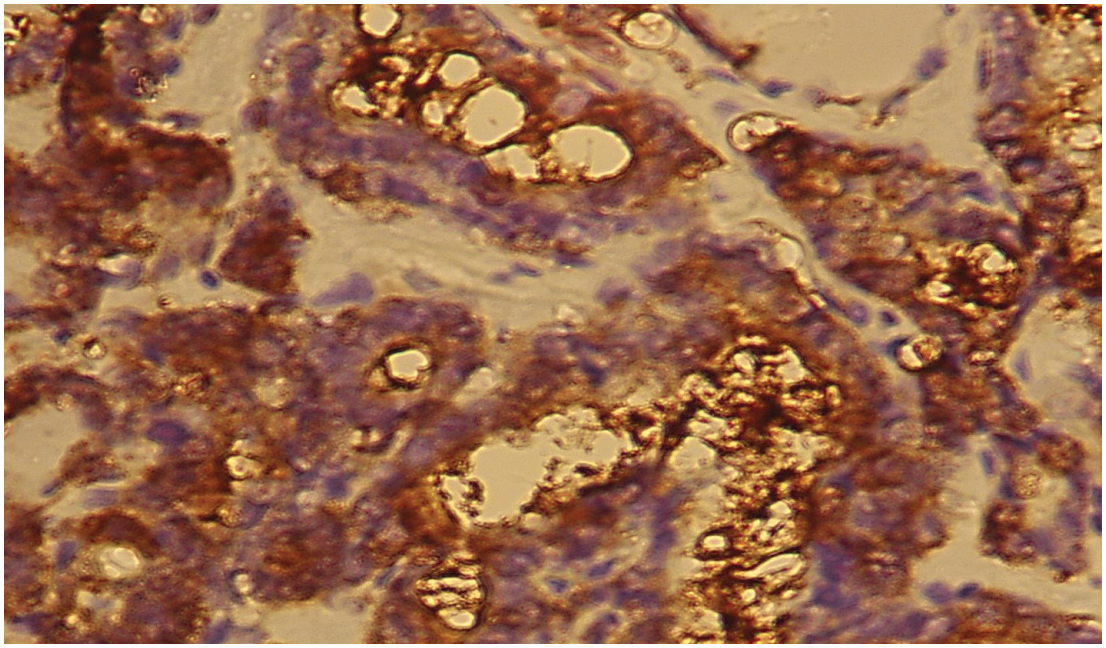

Figure 6 CD56 immunohistochemical staining of thyroid carcinoma applying Avidin-Biotin method. Brown DAB staining indicating positive expression

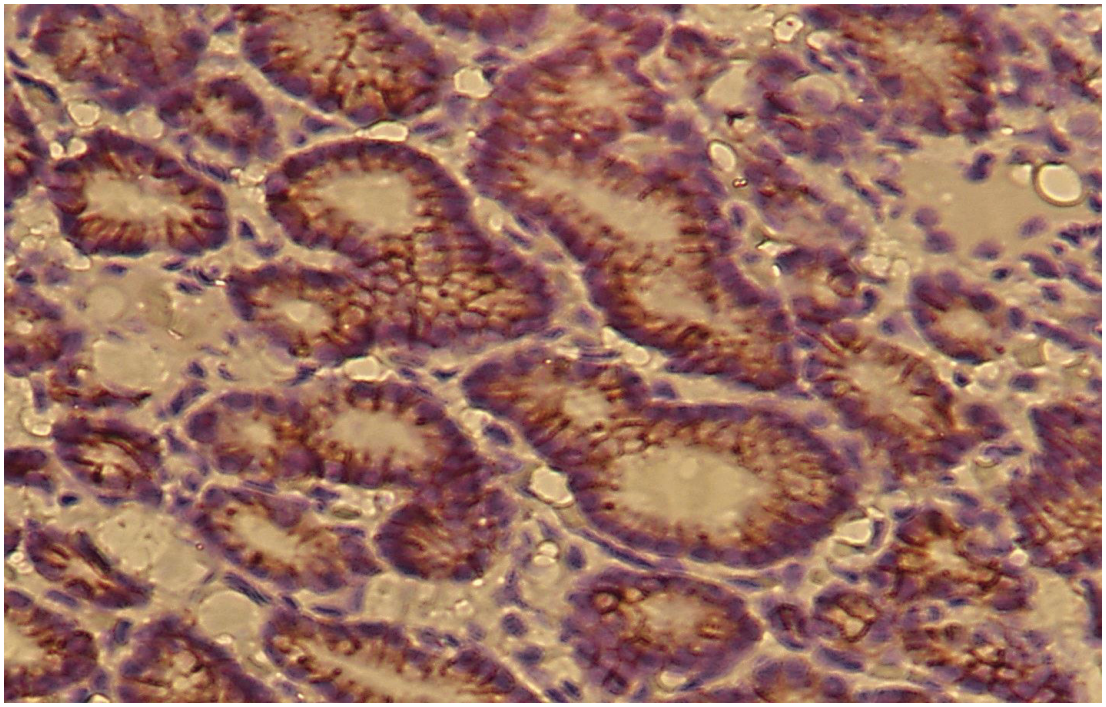

Figure 7 Galectin-3 immunohistochemical staining of thyroid carcinoma applying Avidin-Biotin method. Brown DAB staining indicating positive expression

Combined CK19 and CD56 positive expressions were identified in 9/35 (25.7%), 3/12 (25%) and 3/3 (100%) of the papillary thyroid carcinoma, follicular thyroid carcinoma, and undifferentiated thyroid carcinoma, respectively (Figure 8). Combined CK19 and Gal-36 positive expressions were identified in 25/35 (71.4%), 7/12 (58.3%) and 3/3 (100%) of the papillary thyroid carcinoma, follicular thyroid carcinoma, and undifferentiated thyroid carcinoma, respectively (Figure 9).

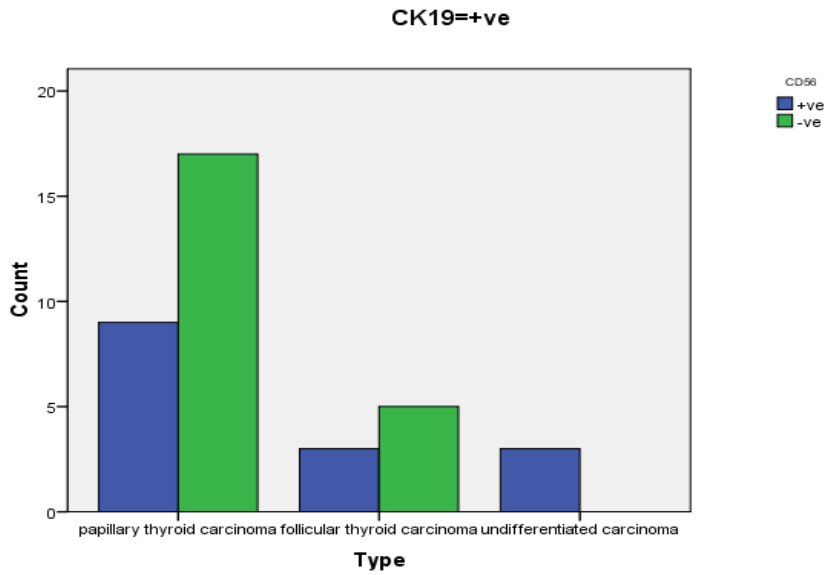

Figure 8 Combined immune-expression of CK19 and CD56 in thyroid carcinoma

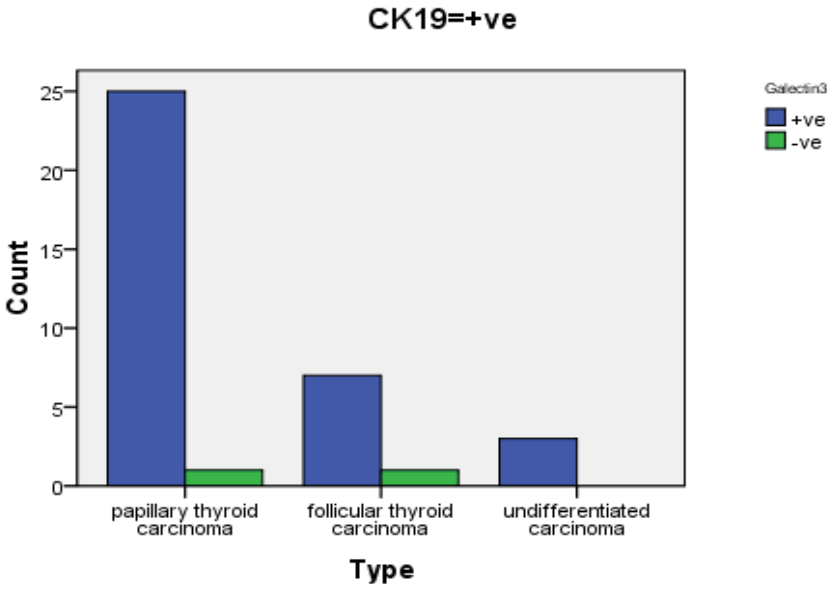

Figure 9 Combined immune-expressions of CK19 and Gal-3 in thyroid carcinoma

DISCUSSION

Thyroid disorders represent a major problem in Saudi Arabia, particularly in the northern part of the country. Several of these thyroid conditions are tumors, a number of them are thyroid carcinomas. Thyroid carcinomas are extremely prevalent among females (90%). The predominance of thyroid carcinoma among females was well documented [13]. Thyroid carcinoma is >2.8 times more frequent among females than males. Although the gender differences in term of the thyroid carcinoma aggressiveness, prognosis, and prevalence are well-established, the reason behind the gender disparity is still equivocal. Reproductive factors and estrogen receptor status were hypothesized to be responsible for the gender disparity in thyroid cancer [14].

Although the majority of thyroid cancers were found to occur at middle age in this study, there was a reasonable number occurring at a younger age <30 years mainly among females. Younger patients were reported to present with more aggressive stages of thyroid cancer, but with favorable prognosis compared to older ones [15]. Some studies suggested that there is no age cut-off affords any unique risk-stratification in patients with thyroid carcinoma [16].

The vast majority of patients in this study presented with papillary thyroid carcinoma (70%). Such findings were previously reported from Saudi Arabia and globally [5,17,18]. Follicular thyroid carcinoma represented 24% in the present study, though the international values ranged from 10% to 40% in respect to iodine status [19,20]. The majority of the cases of papillary thyroid carcinoma were seen in younger age ≤ 30 years, hence, most of the cases of follicular carcinoma were found at the older age <50 years. It was reported that papillary carcinoma was more likely to occur at a younger age <45 years [21], whereas, follicular thyroid carcinoma was reported to affect older people [22]. On the other hand, all cases of undifferentiated thyroid carcinoma were found at older age >50 years [23].

In the current study, CK19 was positively expressed in 74% of the cases of papillary thyroid carcinoma, 66.7% of follicular carcinoma and all cases of undifferentiated thyroid carcinoma. Papillary thyroid carcinoma is acceptable as a consistent tumor marker of this thyroid carcinoma subtype. Though CK19 expression was not linked to demographical and clinicopathological features, the lower expression may favor poor prognosis [24]. Moreover, it was reported that CK19 is a valuable marker for identification of both papillary and follicular thyroid carcinoma. The high expression of this marker usually predicts aggressive behavior particularly in patients with subgroups of thyroid carcinoma with worse prognosis [25].

Positive CD56 expression was demonstrated in 45.7%, 58.3%, and 100% of the papillary, follicular and undifferentiated thyroid carcinomas, respectively. CD56 is a useful marker for the differentiation of the follicular epithelium. Its expression in normal thyroid, benign thyroid lesions, and most follicular tumors and absence of expression in papillary thyroid carcinoma render it as a useful marker to determine the diagnosis of thyroid carcinoma, particularly when applied in combination with other thyroid cancer-related markers [26]. The combined use of CK19 and CD56 is useful in discriminating papillary thyroid carcinoma and its variants from other mimicking thyroid lesions [27].

Positive Gal-3 expression was demonstrated in 71.4%, 58.3%, and 100% of the papillary, follicular and undifferentiated thyroid carcinomas, correspondingly. As Gal-3 marker is involved in cell growth and malignant transformation in various organs including thyroid, its positive expression is significantly higher in thyroid carcinoma compared to benign thyroid neoplasms.

Although the combined expressions of the markers have shown variable values, there was statistically a strong positive expression association between CK19 and Gal-3 ($p<0.05$) in papillary thyroid carcinoma. The positive expression of all 3 markers in undifferentiated may predict the increased expression of the markers with the advancement of the disease.

Though the present study has provided useful data in term of prospective thyroid cancer overall management and research orientation, it has some limitations including the absence of clinical data and patients follow up.

CONCLUSION

Papillary thyroid is the most common thyroid cancer in Northern Saudi Arabia. Females represent more than 90% of the cases of the thyroid carcinoma in Northern Saudi Arabia. CK19, CD56, and Gal-3 are useful for the assessment of thyroid carcinoma. The findings of the present study regarding the assessment of these markers in relation to clinicopathological features might be useful in the future management of thyroid cancer in Saudi Arabia.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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