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Study Of RET/PTC1 Translocation in Differentiated Thyroid Cancer

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ABSTRACT

Background: Thyroid cancer is a very common malignancy of endocrine system with increasing incidence rate over the previous two decades. Most thyroid cancers are well-differentiated papillary carcinomas or follicular carcinomas. Several risk factors have been attributed to the development of thyroid carcinomas such as exposure to ionizing radiations, iodine uptake, and Hashimoto's thyroiditis. In addition to environmental factors, genetic factors are involved in thyroid cancer. RET/PTC translocation plays a role in pathogenesis of differentiated thyroid carcinoma. The most common rearrangement types are RET/PTC1 and RET/PTC3, which are found mainly in papillary thyroid carcinoma.

Objective: To study the association between RET/PTC translocations and differentiated thyroid carcinoma, its clinical implications and the role RET/PTC translocations in tumor genesis and aggressiveness.

Patients and Methods: This prospective study was conducted on 100 patients with provisional diagnosis of nodular goiter underwent thyroidectomy. Detection of RET/PTC1 translocation was studied in our patients within thyroid nodules. After full history taking, examination and investigation for 100 patients, who underwent thyroidectomy postoperative pathology and were 25 malignant 75 benign cases. Specimens from this 100 cases were subjected to RET/PTC1 translocation testing.

Results: The incidence of RET/PTC1 gene translocation was 8 case of 25 cases of malignant cases (32%), and three cases of 75 benign cases (4%).

Conclusion: RET/PTC1, RET/PTC3 translocation plays an important role in the pathogenesis of differentiated thyroid cancer. The RET/PTC1, RET/PTC3 translocations were significantly associated with differentiated thyroid cancer especially papillary variants.

Keywords: RET/PTC translocation, Thyroid Cancer, Total thyroidectomy.

INTRODUCTION

Thyroid nodules are a very common clinical finding; the prevalence of palpable nodules ranges from 4 to 7 % in general population. Although only less than 5% of palpable nodules are malignant lesions, thyroid cancers are the most frequent endocrine malignancy accounting for about 5–10% of thyroid nodules [(1)]. About 95% of malignant lesions are derived from thyroid follicular cells and are distinguished into well differentiated, either papillary (PTC) or follicular (FTC) histotype, and anaplastic thyroid carcinoma [(2)].

Over the last several years, significant progress has been made in understanding the genetic mechanisms of thyroid cancer and in the development of molecular tests for cancer diagnosis in thyroid nodules. RET/PTC translocation plays a role in pathogenesis of differentiated thyroid carcinoma. The most common rearrangement types are RET/PTC1 and RET/PTC3 which are found mainly in papillary thyroid carcinoma [(3)].

A lot has been discovered between the relationship of RET/PTC rearrangements and PTC clinical pathological, and epidemiological features. Although present also in non-irradiated cases, RET/PTC rearrangements are related to radiation exposure and are more frequent in patients with radio induced PTC.

Among all RET/PTC rearrangements, RET/PTC1, and RET/PTC3 are in general the most frequent. RET/PTC3 is much more prevalent in irradiated PTC especially in those with solid variants [(4)].

This research aimed to study the association between RET/PTC translocations and differentiated thyroid carcinoma, its clinical implications and the role RET/PTC translocations in tumor genesis and aggressiveness.

PATIENT AND METHODS

This prospective case series study was conducted during the time between September 2017 and September 2020 in Mansoura Endocrine Surgery Unit, Mansoura University Hospital. The study included 100 cases with provisional diagnosis of nodular goiter who underwent thyroidectomy.

The study included patients with nodular goiter either multinodular goiter (MNG) or solitary thyroid nodule (STN), as assessed by clinical examination, ultrasonography or computed tomography and recurrent goiter.

Patients who had thyrotoxic goiter and undifferentiated carcinoma were excluded from the study, also patients having psychiatric disease, were excluded as well.

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Ethical consent:

An approval of the study was obtained from Mansoura University Academic and Ethical Committee.

Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methodology of RNA extraction and purification:

To semi quantify RET/PTC1, rearrangement, total RNA was purified through Gene JET RNA Purification Kit (K0731, USA), Thermo Scientific.

RNA extraction:

a) Weigh the tissue (use up to 30 mg of fresh or frozen tissue), take the insect and disrupt the material by one of the following methods: a) Disruption using a mortar and pestle. Place up to 30 mg of tissue (use up to 10 mg of papillary thyroid carcinoma tissue) or insect into liquid nitrogen and grind thoroughly with a mortar and pestle. Transfer the tissue or insect powder immediately into a 1.5 mL micro centrifuge tube containing 300 µL of lysis buffer supplemented with β-mercaptoethanol or DTT. Vortex for 10 s to mix thoroughly
b) 600 µl of diluted proteinase K was added (10 µL of the included proteinase K diluted in 590 µL of TE buffer). Vortex to mix thoroughly and incubate at 15-25°C for 10 min.
c) Centrifuge for 5 min at ≥12000 × g. Transfer the supernatant into a new RNase-free microcentrifuge tube.
d) 450 µL of ethanol (96-100%) was added and mix by pipetting.
e) 250 µL of wash buffer 2 was added to the Gene JET RNA purification column and centrifuge for 2 min at ≥12000 × g. optional. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the Gene JET RNA Purification Column to a sterile 1.5 mL RNase-free microcentrifuge tube.
f) 100 µL of water, nuclease-free was added to the center of the Gene JET RNA purification column membrane. Centrifuge for 1 min at ≥12000 × g to elute RNA.
g) The purification column was discarded. Use the purified RNA for downstream applications or store RNA at -20°C or -70°C until use.

DNA construction and gene detection:

Preparation of C-DNA from total extracted RNA for all samples was carried out via Verso 1-Step RT-PCR ReddyMix Kit (Thermo Scientific, AB-1454/LD/A) according to manufacturer protocol with addition of two specific primers (primer listed in table).

Table (1): Sequence of specific primer for RET/PTC1 gene.

	Sequence	Target bp
RET/ PTC1 gene	ATTGTCATCTCGCCGTTC CTTTCAGCATCTTCACGG	306
Positive control	ACAAACTGAAGTGCAAGGCA GCCTTGACCACTACTTTTCCAAA	151

PCR amplification:

Specific genomic fragments were amplified through Gene by Polymerase Chain Reaction (Creacon, Thermo cycler, Holland) system cycler. PCR for amplified genomic DNA was carried out. The reaction consists of 35 cycles consisting of denaturation for 30 seconds at 94°C, annealing for 1 minute at 55°C, and extension for 1 minute at 72°C, followed by a final extension for 7 minutes at 72°C. The product was stored at –20°C or 4°C.

Agarose gel electrophoresis and detection of the amplification products:

1.5% agarose solution was prepared by adding 0.75g agarose to 50 ml of 1x TBE electrophoresis buffer in 50 ml flask. Heating in a microwave oven then dissolved the agarose. The agarose was cooled in 50°C.

A comb was inserted in electrophoresis bed and the agarose was poured in it. Great care should be taken during pouring of the agarose to avoid bubbles formation. The gel solidified within 15 min and became cloudy, the electrophoresis apparatus was filled with the electrophoresis buffer and the comb was removed creating 6 or 10 wells for sample application. Electrodes were connected to the power supply and the later was turned on. It was adjusted at 80 Volts for 100 min. The gel was removed from its bed and transferred to the gel staining tray for staining with ethidium bromide for 30 minutes followed by 20 minutes distain in distilled water. Specific DNA bands were eluted from agarose gel.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) or Fisher's exact test was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation), and range. P value < 0.05 was considered significant.

RESULTS

Demographic data and results of postoperative pathology in the study population are shown in table 2.

Table (2): Demographic and results of postoperative

pathology in the study population.

	Study group (n=100)
Age/y	
Mean ± SD	43.88±14.47
Min-Max	18-78
Sex	
Male	18 (18%)
Female	82 (82%)
Postoperative pathology:	
Benign (75)	
Colloid goiter	59 (59%)
Lymphocytic thyroiditis	7 (7%)
Follicular adenoma	9 (9%)
Malignant (25)	
PTC	18 (18%)
FVPTC	4 (4%)
F.C	3 (3%)

32% of cancer cases (8 cases) were associated with lymph node metastasis. 40% of them had capsular invasion. 24% of malignant tumors (6 cases) were associated with extrathyroidal extension (ETE) and one of them infiltrated the trachea and the esophagus. One case (5%) was associated with pulmonary metastasis (Table 3).

Table (3): Comparison of sign of aggressiveness according to nature

Variables	Malignant group (n=25)	Benign group (n=75)	P-value
LNs metastasis			
No	17 (68%)	75 (100%)	<0.001 *
Yes	8 (32%)	0 (0%)	
Capsular invasion			
No	15 (60%)	75 (100%)	<0.001 *
Yes	10 (40%)	0 (0%)	
Extrathyroid extension			
No	19 (76%)	75 (100%)	<0.001 *
Yes	6 (24%)	0 (0%)	
Perineural invasion			
No	22 (88%)	75 (100%)	0.014*
Yes	3 (12%)	0 (0%)	

RET/PTC1 rearrangement analysis in pathological samples from removed thyroid tissue revealed 8 cases of malignant group and 3 cases of benign group positive for RET/PTC1 gene translocation (Table 4).

Table (4): Comparison of RET/PTC 1 according to nature of the lesion.

Variables	Malignant group (n=25)	Benign group (n=75)	P-value
RET/PTC 1			
Negative	17 (68%)	72 (96%)	<0.001*
Positive	8 (32%)	3 (4%)	

There was no significant difference between gene +ve and gene -ve as regard lymph nodes metastasis, capsular and perineural invasion, and Bethesda classification (Table 5).

Table (5): Correlation between RET/PTC 1 gene and tumor aggressiveness.

Variables	Gene +ve group (n=8)	Gene -ve group (n=17)	P-value
Lymph nodes metastasis			
No	4 (50%)	13 (76.47%)	0.193
Yes	4 (50%)	4 (23.53%)	
Capsular invasion			
No	3 (37.5%)	12 (70.59%)	0.128
Yes	5 (62.5%)	5 (29.41%)	
Extrathyroid extension			
No	6 (75%)	13 (76.47%)	0.651
Yes	2 (25%)	4 (23.53%)	
Perineural invasion			
No	7 (87.5%)	15 (88.24%)	0.704
Yes	1 (12.5%)	2 (11.76%)	
Bethesda class			
III	4 (50%)	5 (29.41%)	0.491
IV	2 (25%)	7 (41.17%)	
V	2 (25%)	5 (29.41%)	

Patient with differentiated thyroid cancer (papillary variants) (Figure 1):

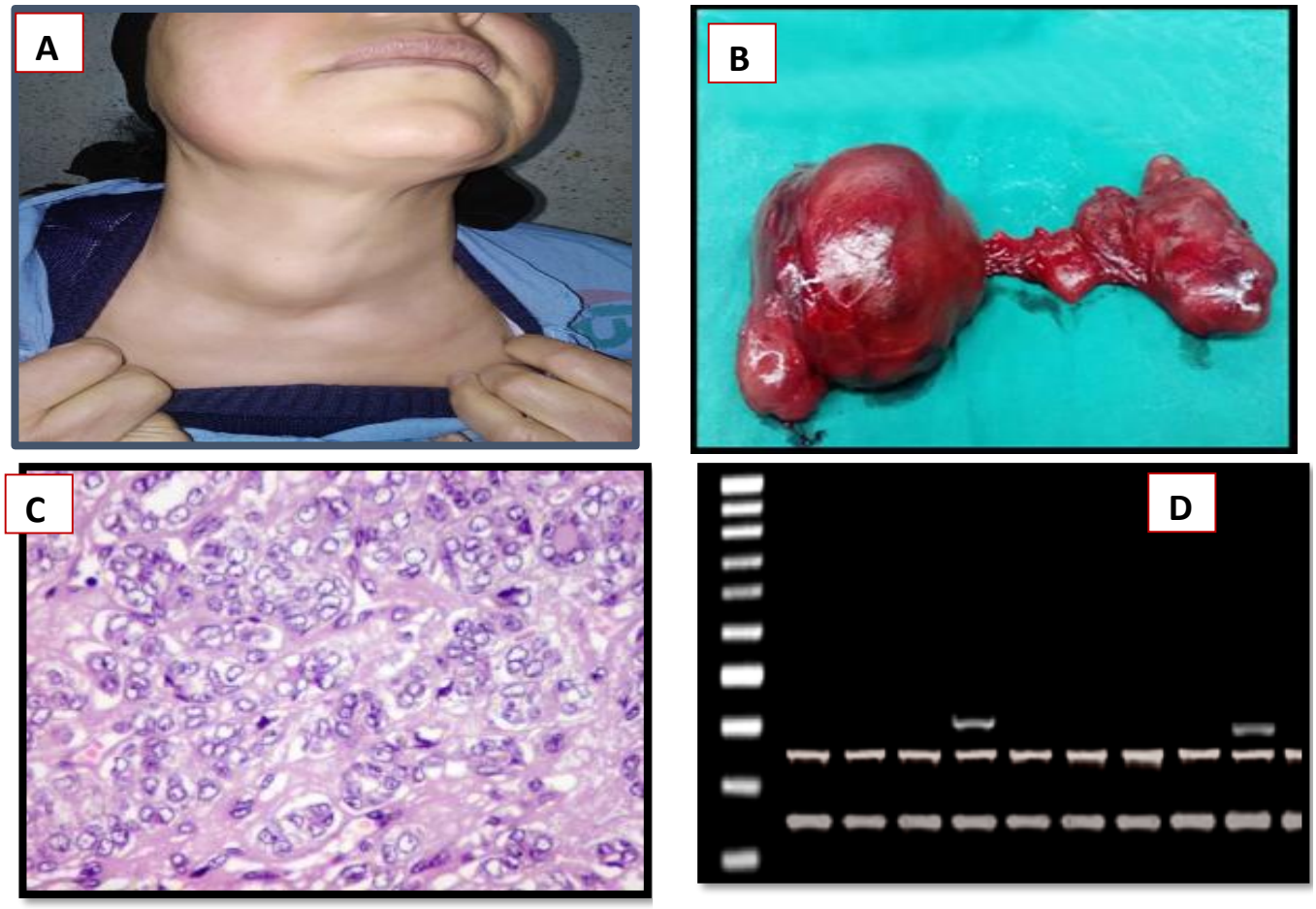

Fig. (1) : (A) female patient with clinically solitary large right thyroid nodule (B) removed thyroid specimen is shown. (C) Pathological examination proved to be follicular variant of papillary thyroid carcinoma. (D) Positive agarose gel electrophoresis for RET/PTC gene translocation.

Patient with simple multinodular goiter (Figure 2):

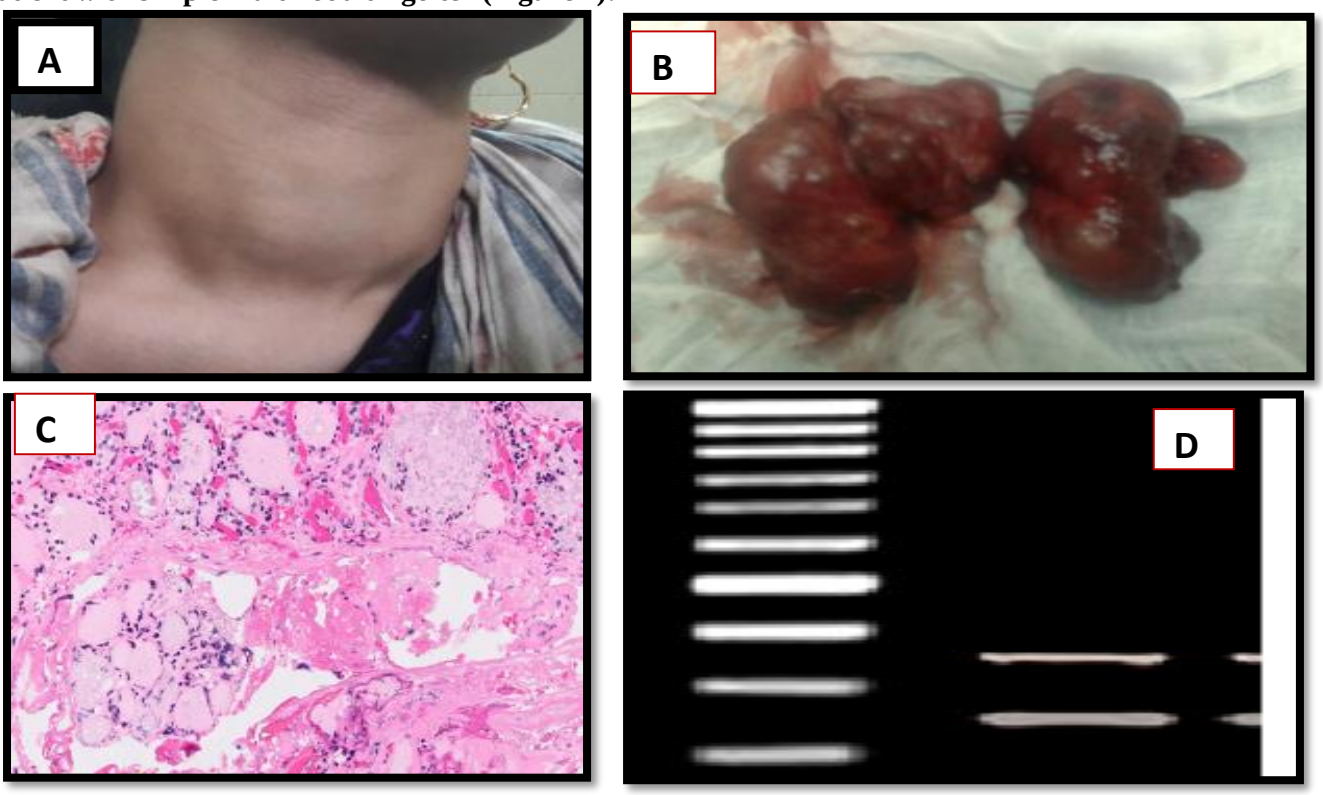

Fig. (2): (A) Female patient with multinodular (B) removed thyroid specimen (C) Pathological examination proved to be colloid goiter (D) negative agarose gel electrophoresis RET/PTC gene translocation.

Patient with thyroiditis (Figure 3):

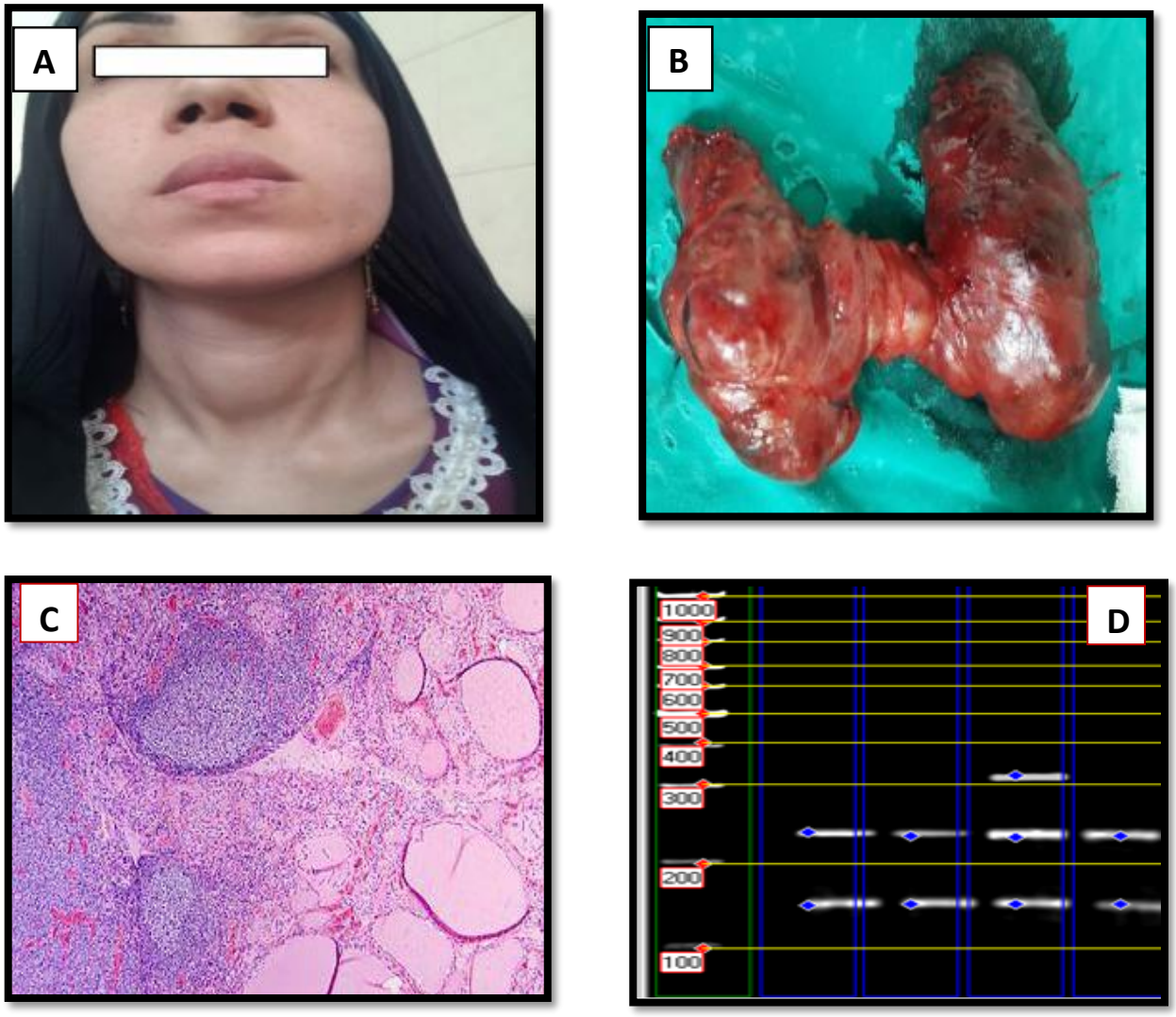

Fig. (3): (A) Female patient with diffuse goiter, (B) removed thyroid specimen (C) Pathological examination proved to be Hashimoto's thyroiditis (D) positive agarose gel electrophoresis for RET/PTC gene translocation case No 3 in column.

DISCUSSION

Thyroid carcinoma is the most frequent endocrine cancer accounting for 5–10% of thyroid nodules. Papillary histotype (PTC) is the most prevalent form accounting for 80% of all thyroid carcinoma. Although much is known about its epidemiology, pathogenesis, clinical, and biological behavior, the only documented risk factor for PTC is the ionizing radiation exposure. Rearrangements of the Rearranged during Transfection (RET) proto-oncogene are found in PTC and have been shown to play a pathogenic role [(5)].

This study was conducted at Mansoura university Hospitals aiming to find the correlation between RET 1 gene and malignant thyroid lesions. We included 100 cases who were divided into two groups; (75) benign and (25). There was no significant difference between the two groups regarding age (p = 0.832), as the mean age of the included cases was 43.14 and 44.32 years in the malignant and benign groups respectively. Similar to our results, other authors reported no significant difference between benign and malignant cases regarding age of the participants (p = 0.347). The mean age of both groups was 58 and 50 years respectively [(6)].

In the current study, females represented 84 and 80% of cases in the malignant and benign groups respectively. There was no significant difference between the two groups regarding that parameter. In most studies, age and sex were not associated with malignancy [(7-9)].

In the current study, there was a significant difference between the two groups regarding ATA classification system (p = 0.009). High grade suspicion was reported in 40% of malignant lesions, while it was not reported in the benign group. In addition, low grade suspicion was the commonest reported category in the benign group (56%). Likewise, another study reported that malignant lesions were distributed along high suspicion (50%) and intermediate suspicion (50%). Conversely, most of the benign lesions were

reported to have very low or intermediate suspicion [10].

When it comes to the detection of both RET/PTC1 in our study, they were more significantly expressed in malignant cases compared to benign ones. RET/PTC1 mutation was detected in 32% of malignant cases, while it was only detected in 4% of benign cases (p= 0.001).

The frequency of RET/PTC rearrangements (average 25% of the cases, and this coincides with our findings) varies considerably in different patient series [11]. Other study reported that clonal RET/PTC rearrangements occur in about 20% of PTC and are specific for this tumor [12]. Studies done on Korean population found RET/PTC1, RET/PTC2 and RET/PTC3 rearrangements to be 6.5%, 6.5% and 0% respectively whereas studies from Japanese population showed prevalence of RET/PTC rearrangements to be 30% in PTC [13]. RET/PTC rearrangements have been reported to be very high (69% to 83%) in areas exposed to radiations [14].

The heterogenicity between different reports may depend on patients' exposure to different etiologic factors. As an example, in pediatric patients and in cases from areas contaminated by radioiodine isotopes, RET/PTC frequency can reach 50%–70% [12]. Furthermore, multiple studies have established the link between this specific mutation and tumor aggressiveness. In a recent study, RET/PTC1 positivity was also correlated with various clinic pathological characteristics. RET/PTC1 rearrangements were insignificantly associated with gender, lymph node metastasis or elevated TSH levels [15]. **Adeniran *et al.*** [4] reported that lymph node metastasis is significantly associated with RET/PTC rearrangements.

CONCLUSION

From our study it has shown that RET/PTC1, RET/PTC3 translocation play an important role in the pathogenesis of differentiated thyroid cancer. The RET/PTC1, RET/PTC3 translocations were significantly associated with differentiated thyroid cancer especially papillary variants. RET/PTC translocation can be used as biomarkers for development of targeted therapy for differentiated thyroid cancer.

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Conflict of Interest: Nil.

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