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Immunohistochemical Expression Of P53 In Iraqi Patients Suffered From Papillary Thyroid Carcinoma (Cross-Sectional Study)

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Abstract

Thyroid cancer has been continuously increasing in recent years (at least 4% annually) and is the fastest-growing cancer in the United States, doubling in the last three decades.
Tall cells, columnar cells, diffuse sclerosing, solid/trabecular, and insular types of papillary thyroid cancer are all more aggressive than ordinary papillary thyroid tumors.
Despite the high prevalence of thyroid cancer and the availability of HPV vaccines for cancer prevention, thyroid cancers have little experiences with HPV infections.

Introduction

Thyroid cancers make up about 95 percent of all endocrine tumors, accounting for about 2.5 percent of all cancers.
Thyroid cancer is estimated to cause 52,890 instances in the United States in 2020, with over 2180 indivi duals (4.1 percent) dying from the disease.
Thyroid cancer has been steadily increasing in recent years (at least 4% yearly) and is the fastest growing cancer in the United States, having doubled in the last three decades [(1)].
Tall cells, columnar cells, diffuse sclerosing, solid/trabecular, and insular types of papillary thyroid carcin oma (PTC) are all more aggressive than ordinary papillary thyroid tumours.
Thyroid cancer instances had roughly 79 percent papillary carcinomas and 13 percent follicular carcinom as [(2)]. Recent studies have revealed the fundamental role played by signaling routes such as MAPK and

PI3K - AKT in thyroid cancer pathogenesis. The activation and interaction of these routes, often in relation and coordination, constitute the primary oncogenic mechanism that produces thyroid cancer. These oncogenic alterations play a key role in the revolution and growth of thyroid cancer. There have also been increasingly identified secondary molecular derangements that are induced by the over-activation of these pathways, Oncogenes synergize with each other and enhance thyroid cancer signalling [3].

Papillary thyroid carcinoma (PTCs) with this mutation occurs in approximately 45% of all cases [4]. In PTCs, there are a few uncommon BRAF mutations that alter nucleotides around codon 600 and activate the BRAF kinase [5,6]. The results of a prior comprehensive multicenter study demonstrated that BRAFV600E is associated with poor clinic-pathological outcomes of PTC, such as aggressive pathological features, increased recurrence, and loss of radioiodine avidity [7].

The prevalence of PTC was found to be 4.5% in 2019 in a study conducted in Duhok, Kurdistan region, Iraq [8]. According to the Iraqi cancer registry, 52,03% of thyroid gland cancers were PTC and NOS, 11.05% were papillary microcarcinoma and 4.55% were follicular variant of papillary carcinoma in 2018 [9].

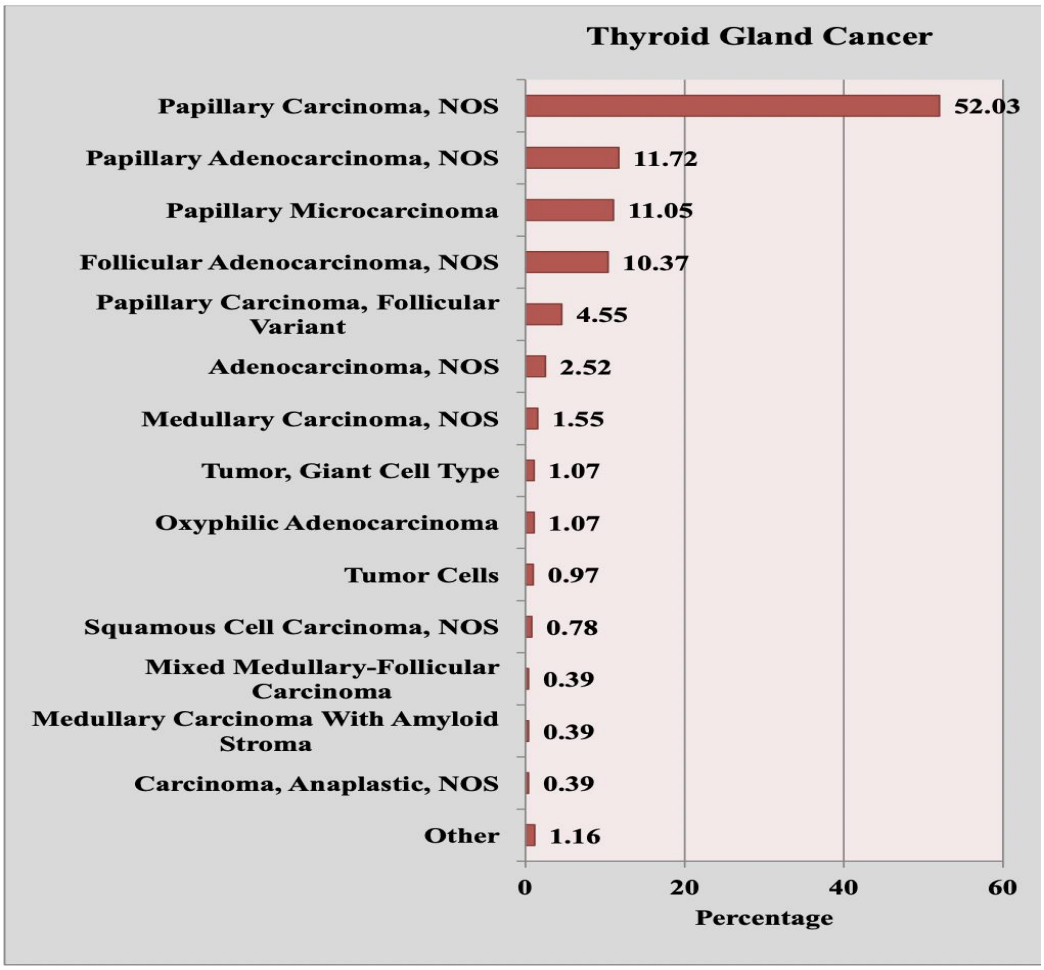

Figure (1) Thyroid Gland cancer in females by the type of morphology, Iraq, 2018 [9]

Viral persistent infections may cause chronic inflammation that is characterized by the release and/or expression of inflammatory cytokines. Reactive oxygen and nitrogen species (RONS) are also involved in chronic inflammation [10]. Induced chronic inflammation suppresses anti-tumor immunity and may promote tumor progression and metastasis [11, 12, 13]. Tumor growth is enhanced by chronic inflammation in several ways, including growth factor secretion, angiogenesis, and tissue remodelling [14]. Cytokines such as transforming growth factors beta (TGFβ-), interleukin (ILs), and tumor necrosis factor (TNF) may accelerate the proliferation and invasion of breast and thyroid cancer cells. (TNF-α) [15, 13, 16, 10]. Inflammation is also mediated by NF-*B and RONS, which play a crucial part in initiating and developing solid tumours [17, 16].

Material and methods

This study incorporates a total of 66 samples of papillary thyroid cancer. Formaldehyde was used to preserve the tissue, which was then embedded in paraffin blocks.

Sample Size = $\frac{(\mathbf{Z1-a/2})\mathbf{2\,P(1-P)}}{\mathbf{D2}}$

Here:

$\mathbf{Z_1}$. $a_{/2}$ = Is standard normal variate (at 5% type 1 error (P <0.05) it is 1.96 and at 1% type 1 error (P < 0.01) it is 2.58. as in majority of studies P values are considered significant below 0.05 hence 1.96 is used in formula.

P = Expected proportion in population based on previous studies or pilot studies.

D = Absolute error or precision – Has to be decided by researcher.

When Statistical equation

- sample size = $\frac{\mathbf{1.96^2*0.045*(1-0.045)}}{\mathbf{0.05^2}}$ = 66 patient.

formaldehyde conserved submersed blocks of malignant specimens were used from breast carcinoma was used as control for immunohistochemistry.

Dako monoclonal mouse antihuman P53 protein marker was used [18]:

Clone: DO-7

Code number: M7001

Immunogen recombinant human wild -type P53 protein

Isotype: IgG2b, kappa

Dilution: Ready to use

Data were summarized as tables and expressed in frequency and percentages; The results were analyzed using IBM SPSS Statistics for Mac (version 26). The numerical variables are shown as means and standard deviations obtained by applying the independent t test. Chi square test was used to find association between different categorical variables, $P<0.05$ was regarded as a statistically significant value.

Results

When applying the Qui-square statistics on age, gender and immunohistochemical expression data, the following results were obtained:

Clinico-pathological variables		NO.	%
Age group	Below 45	49	74.2
	Above 45	17	25.8
Gender	Male	15	22.7
	Female	51	75.8
	P53 +	7	10.6
	P53 -	59	87.9

Table (1): Clinicopathological variables

Qui-square statistics were applied on age, gender and immunohistochemical expression data and age data were divided into two groups (above and below 45 years), and the following results were obtained:

Age groups	P53		P-value
	Positive (n=) No. (%)	Negative (n=) No. (%)	
Below 45	3 4.5%	46 69.7%	0.026
Above 45	4 6.1%	12 18.2%	
Male	1 1.5%	14 21.2%	0.00001
Female	6 9.1%	44 66.7%	

Table (2): Immunohistochemical expression of P53 in relation to clinico-pathological variables using Qui-square

Immunohistochemical expression of P53 is significantly affected by the clinicopathological variants (age and gender) with a high percentage of females. Gender was not a factor that affected the positivity of

HPV expression as well (according to Qui-square statistics). While when t independent test was applied: no significance was found for the age factor to be associated with P53 positivity.

	Marker Marker		N	Mean	Std. Deviation	p-value
Age	P53	negative	59	37.2373	11.66832	0.1
		positive	7	43.7143	13.69567	

Table (3): Immunohistochemical expression of P53 in relation to clinico-pathological variables using independent sample t test.

			P53		Total	P-value
			Negative	Positive		
Staging	1	Count	42	6	48	0.7
		% Of Total	63.6%	9.1%	72.7%	
	2	Count	3	0	3	
		% Of Total	4.5%	0.0%	4.5%	
	3	Count	9	1	10	
		% Of Total	13.6%	1.5%	15.1%	
	4	Count	5	0	5	
		% Of Total	7.6%	0.0%	7.6%	
Total		Count	59	7	66	
		% Of Total	89.4%	10.6	100.0%	

Table (4): Association of P53 with PTC staging

No significance was found between P53 expression in papillary thyroid carcinoma and tumor staging.

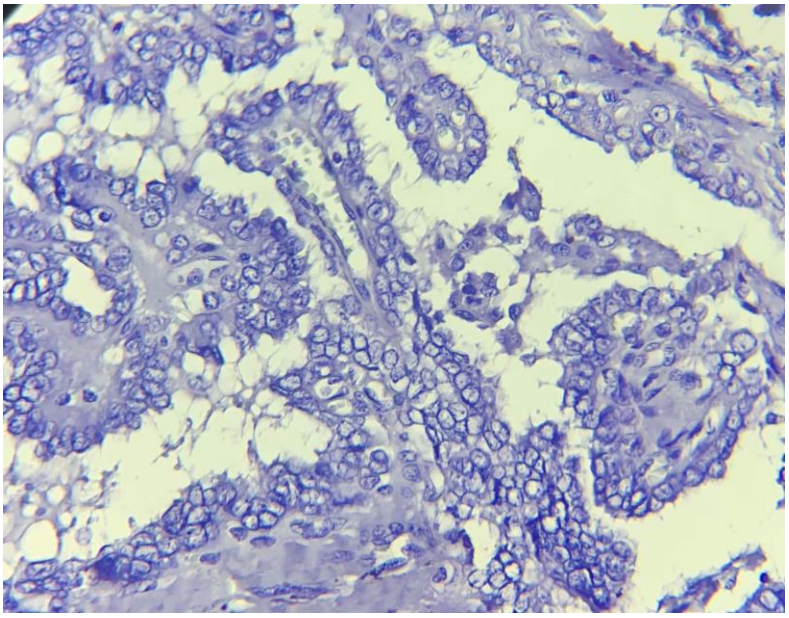

Figure (1): Papillary thyroid carcinoma negative stain for P53 IHC 10x40

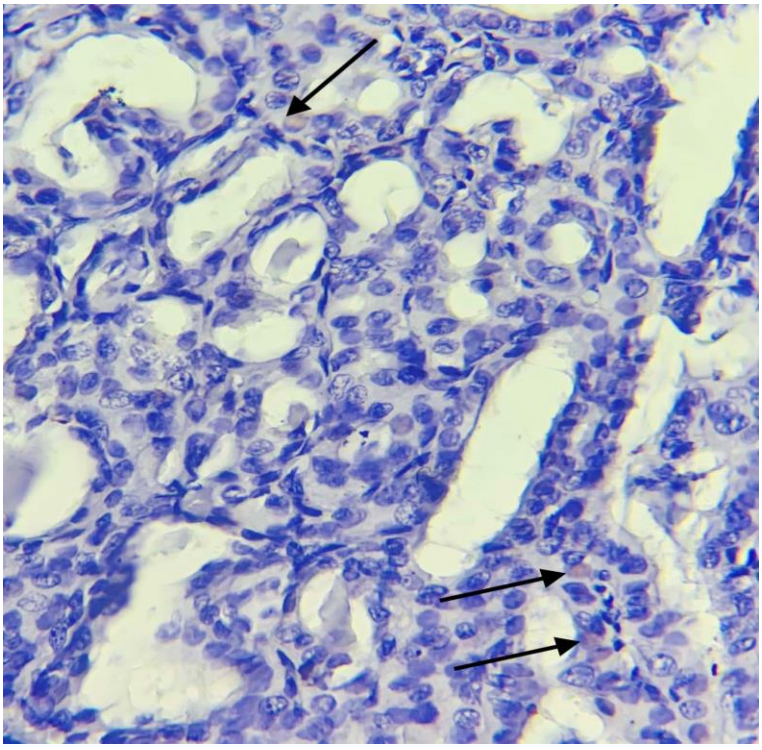

Figure (2): Papillary thyroid carcinoma scattered cells weak positive nuclear stain (arrows) for P53 IHC 10x40

Discussion

Overexpression of the P53 protein was detected in 11 to 59 percent of PTC patients [19]. 10.6% of the samples, however, showed positive P53 results and significant association with age and gender (P value is 0.026 and 0.00001 respectively) according to Qui-square statistics. This agrees with a study conducted in Japan, revealed that P53 protein expression in the primary tumor of PTC was found to be significantly correlated [20].

When applying the independent sample t test on age variable and linking that to P53 results, again a contradicting outcome was found as compared to Qui-square method and age was found to be a nonsignificant factor in relation to P53 over-expression (p-value = 0.1).

A study by Shin MK et al. indicated that aging, sex, tumor stage, recurrence, lymph node cancers, and additional thyroid extension were found to have no significant connection with overexpression of P53 protein [19].

P53 overexpression was not significantly associated with PTC staging although 9.1% of samples were positive for P53 and in stage1. this disagrees with a study by Chen BK et al. which indicated that P53 protein accumulation in PTC tends to be associated with a high frequency of lymph node metastasis, increased tumor size and advanced pathological stage [21].

A study conducted in in Barcelona by C Zafon et al. showed that Mutations in the p53 gene are more common in anaplastic and poorly differentiated carcinomas, and they constitute a late genetic event in thyroid carcinogenesis [22]. This agrees with our study since we only studied PTC samples.

In conclusion: there was no relation between P53 overexpression and PTC since we encountered only early stages PTCs in our study.

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