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

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Abstract

The range of thyroid cancer has been increasing steadily lately (the increase is at least 4% annually) and is the fastest-rising cancer in the U.S.A, doubling in the past three decades. PTC has a wide range of histological variants, such as tall cells, columnar cells, diffuse sclerosing, solid/trabecular, and insular variants that are all more aggressive than conventional papillary thyroid cancers. Despite a closely high range of thyroid cancer and the obtainability of HPV vaccines to prevent cancer, thyroid cancers have few experiences to figure out the importance of HPV infections.

Introduction

Approximately 95% of all endocrine tumours are thyroid cancers, which account for roughly 2.5% of all malignancies. In 2020, the United States is expected to have 52,890 cases of thyroid cancer, and almost 2180 patients (4.1%) will pass away from thyroid cancer. The range of thyroid cancer was increasing stably lately (the increase is at least 4% annually) and is the fastest-rising cancer in the U.S.A, doubling in the past three decades [(1)].

Papillary thyroid carcinoma (PTC) has a wide range of histological variants, such as tall cells, columnar cells, diffuse sclerosing, solid/trabecular, and insular variants that are all more aggressive than conventional papillary thyroid cancers. There are around 79% papillary carcinomas and 13% follicular carcinomas in thyroid cancer cases [(2)].

Many studies have failed to determine the cause of the malignancy of thyroid. Radiation exposure, high iodine intake, auto-immune thyroid disease, and predisposition of genetics are possible risk factors.

Despite a closely high range of thyroid cancer and the obtainability of HPV vaccines to prevent cancer, thyroid cancers have few experiences to figure out the importance of HPV infections [3].

Human papillomavirus is an uncovered Deoxyribonucleic acid virus belonging to the Papillomavirus family. There were over one hundred fifty Human papilloma virus serotypes. As such, their focus differs for contaminating squamous epithelium at various sites, causing various kinds of Human papilloma virus infection (e.g., usual, plantar, palmar, anal, and genital) [4] [5].

They are divided into two groups, according to their cause of tumour development; lower-hazard and higher-hazard, as follows: sixteen, eighteen, thirty-one, thirty-three, forty-five, fifty-two, and fifty-eight. Kinds sixteen and eighteen are often associated with cervix tumours, but they are also linked to other types of tumours. Among Americans, invasive tumours of the cervix, anus, vulva, vagina, phallus, penis, and ovaries are caused by Human papillomavirus kinds sixteen and eighteen in sixty-six percent of oropharyngeal tumours annually. The lower-risk types were usually associated with non-malignant lesions, like verrucae (especially six and eleven types), and may also cause respiratory papillomatosis relapse [6].

Type 16 and 18 are the highest risk of HPV kinds. HPV-related to head and neck carcinomas share molecular characteristics with lung squamous carcinoma, while HPV + linked to head and neck carcinomas, they share the same characteristics with cervical neoplasia [7].

Human papilloma virus (HPV) types with high cancer risk express two oncogenic proteins, E6 and E7. By repressing these genes, HPV-mediated carcinogenesis is impaired and malignant phenotypes are reversed [8]. E6 promotes degradation of the tumour suppressor p53 and increases telomerase expression, it binds to a very important regulatory factor: BAK34. Two essential consequences result from this interaction: cellular resistance to apoptosis and increased chromosomal instability [9]. Oncogenic E7 appears to inhibit apoptosis, progression of cell cycle, as genetic changes accumulate, and the integrity of viruses by binding and inactivating members of the retinoblastoma susceptibility protein (pRb) family leads to uncontrolled cell proliferation [3,10]. RB binds to E2F1, a factor of transcription that prevents it from regulating cellular transcription [11,12].

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 [13]. Induced chronic inflammation suppresses anti-tumour immunity and may promote tumour progression and metastasis [14, 15, 16]. Tumour growth is enhanced by chronic inflammation in several ways, including growth factor secretion, angiogenesis, and tissue remodelling [17]. Cytokines such as transforming growth factors beta (TGFβ-), interleukin (ILs), and tumour necrosis factor (TNF) may accelerate the proliferation and invasion of breast and thyroid cancer cells. (TNF-α) [18, 16, 19, 13]. Inflammation is also mediated by NF-*B and RONS, which play a crucial part in initiating and developing solid tumours [20, 19].

Materials and methods

This current study is a cross sectional one carried out in university of Kufa/ Faculty of Medicine/ Pathology and Forensic Medicine Department. The work was performed in middle Euphrates cancer research unit in period extending from November - until December - 2021. All the cases in this study were gathered randomly from several private pathology laboratories in the Al Najaf governorate. All samples were re-examined by specialist pathologist to confirm the diagnosis. Clinicopathological data were obtained from the relevant histopathological reports available with the tissue specimens, which consisted of patient's age, gender, type of biopsy and final diagnosis.

Anti-HPV16 E6/18 E6 Antibody (C1P5) sc-460 marker was used [21]:

Clone: E6 oncoprotein

Isotype: IgG1, kappa

Dilution: 1:1000

The data were analysed using IBM SPSS Statistics for Mac (version 26), which were presented as tables and expressed in frequency and percentages. The means and standard deviations of the numerical variables are calculated using the independent t-test. The Chi square test was performed to look for correlations between categorical variables, and P value=0.05 was considered statistically significant.

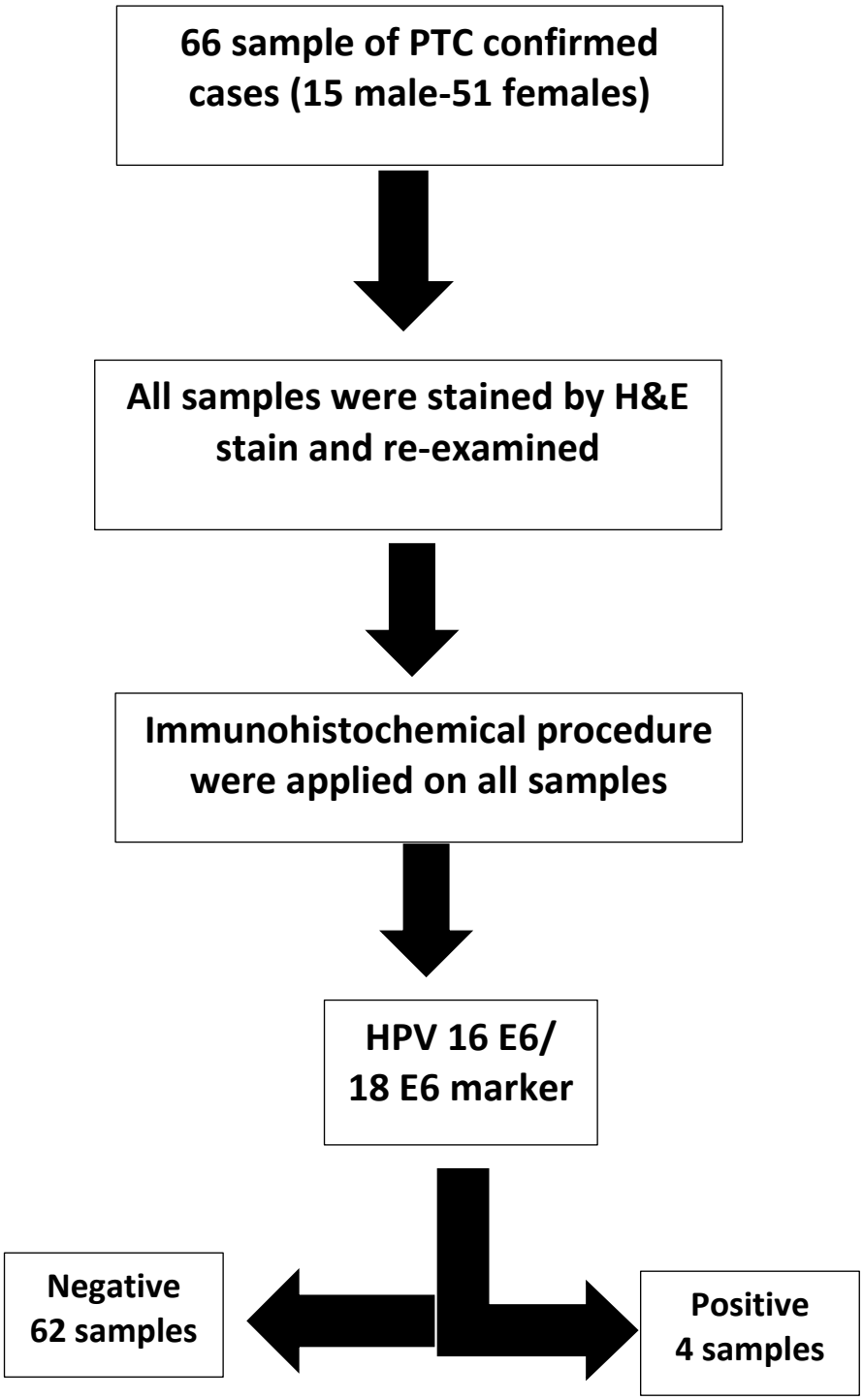

66 sample of PTC confirmed cases (15 male-51 females)
All samples were stained by H&E stain and re-examined
Immunohistochemical procedure were applied on all samples
HPV 16 E6/ 18 E6 marker
Negative 62 samples
Positive 4 samples

Figure (1): diagram showing summary of this study

Results:

Most of the age group included in this study was below 45 years of age (74.2%). Majority of patients included in this study were females, (75.8%). 100% papillary thyroid masses were incorporated in our study confirmed by histopathological readings.

In an immunohistochemical study for all masses 6.1% (4 samples) were positive, 93.9% (62 samples) were negative for HPV.

Age groups	HPV		P-value
	Positive (n=) No. (%)	Negative (n=) No. (%)	
Below 45	3 4.5%	46 69.7%	0.9
Above 45	1 1.5% 1.5%	16 24.2%	
Male	1 1.5%	14 21.2%	0.91
Female	3 4.5%	48 72.7%	

Table (1): Immunohistochemical expression HPV in relation to clinico-pathological variables using Qui-square

No significance has been noticed between immunohistochemical expression of HPV and clinicopathological variants (age and gender). Gender was not a factor that affected the positivity of HPV expression as well (according to Qui-square statistics).

However, by applying the independent sample t test on the age data that took the mean age for all patients collectively, the mean age for the 4 positive samples was found to be 49 ± 13.73560 (standard deviation). That was just significantly associated with increased expression of HPV.

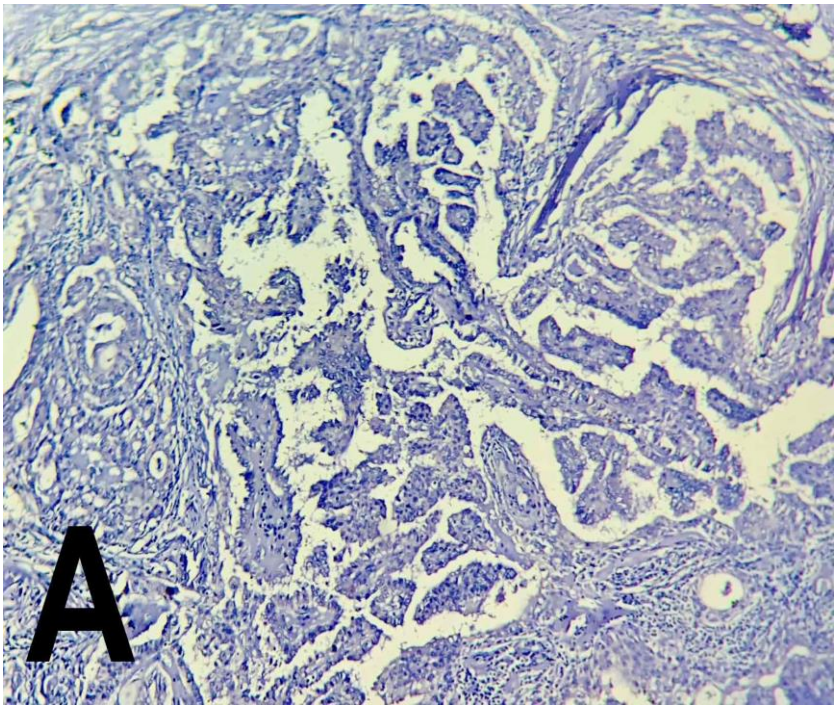
Age	Marker Marker		N	Mean	Std. Deviation	p-value
Age	HPV	negative	62	37.2097	11.59160	0.055
		positive	4	49.0000	13.73560	

Table (2): Immunohistochemical expression of P53 and HPV in relation to clinico-pathological variables using independent sample t test.

In total 72.7% (48 samples) were in stage 1, 68.2% (45 samples) of which were negative for HPV. while only 4.5% (3 samples) were found to be positive for HPV. 13.6% (9 samples), were in stage 3 and negative for both HPV with only 1.5% (1 samples) positive for HPV marker. 7.6% (5 samples) were in stage 4 and only 4.5% (3 samples) were in stage 2 of tumour development. No significance was found between both HPV expression in papillary thyroid carcinoma and tumour staging.

			HPV		Total	P-value
			Negative	Positive		
Staging	1	Count	45	3	48	0.85
		% Of Total	68.2%	4.5%	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	62	4	66	
		% Of Total	93.9%	6.1%	100.0%	

Table (3): Association of HPV with PTC staging



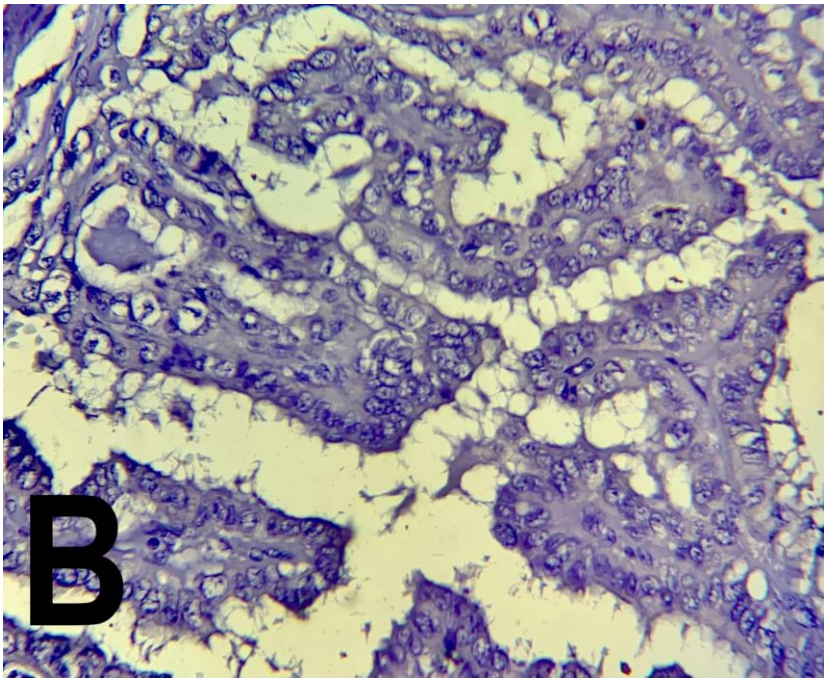


Figure (1): Papillary thyroid carcinoma negative for HPV 16 E6/ 18 E6 IHC. A 10x10. B 10x40

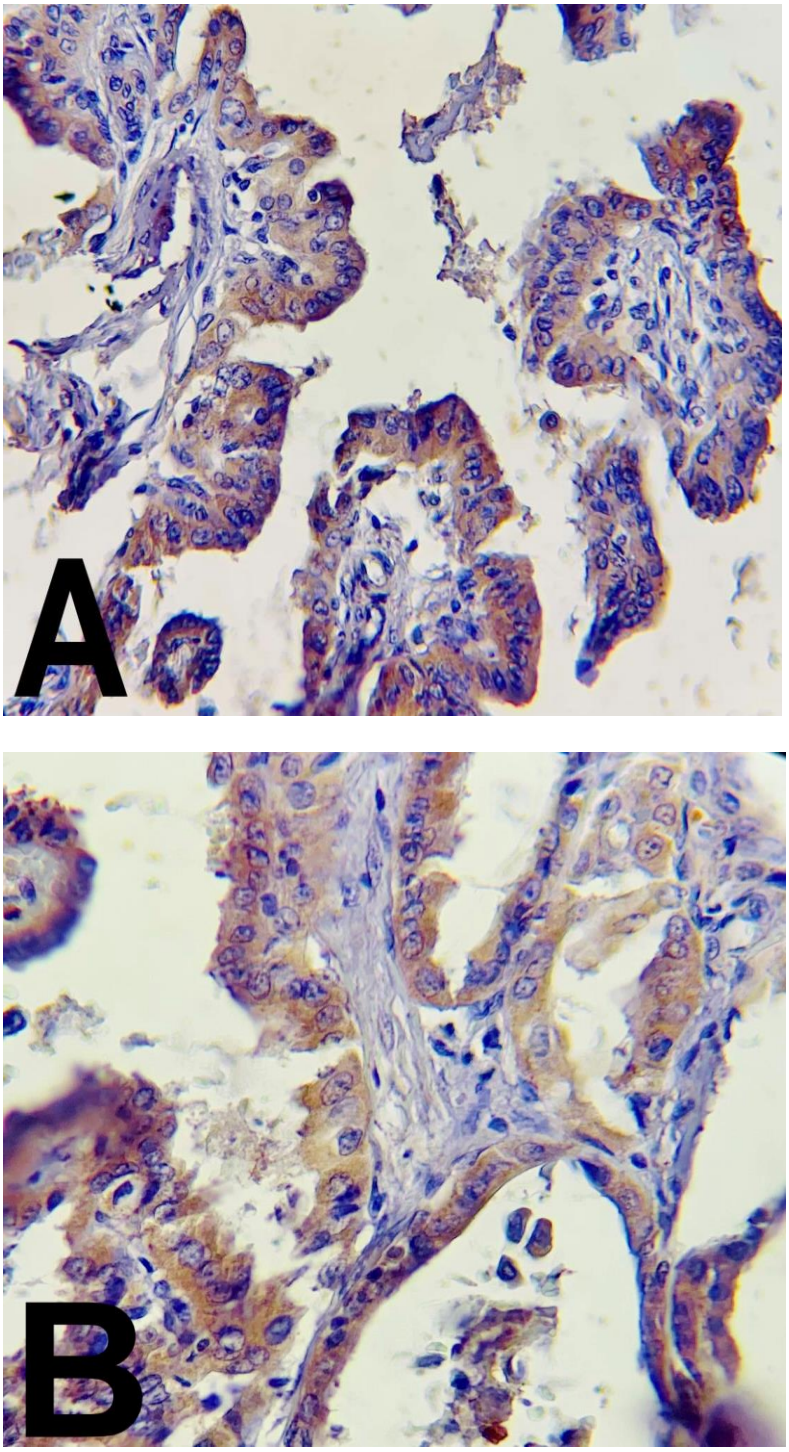


Figure (2): Papillary thyroid carcinoma positive moderate intensity cytoplasmic stain for HPV 16 E6/ 18 E6 IHC. A 10x10. B 10x40

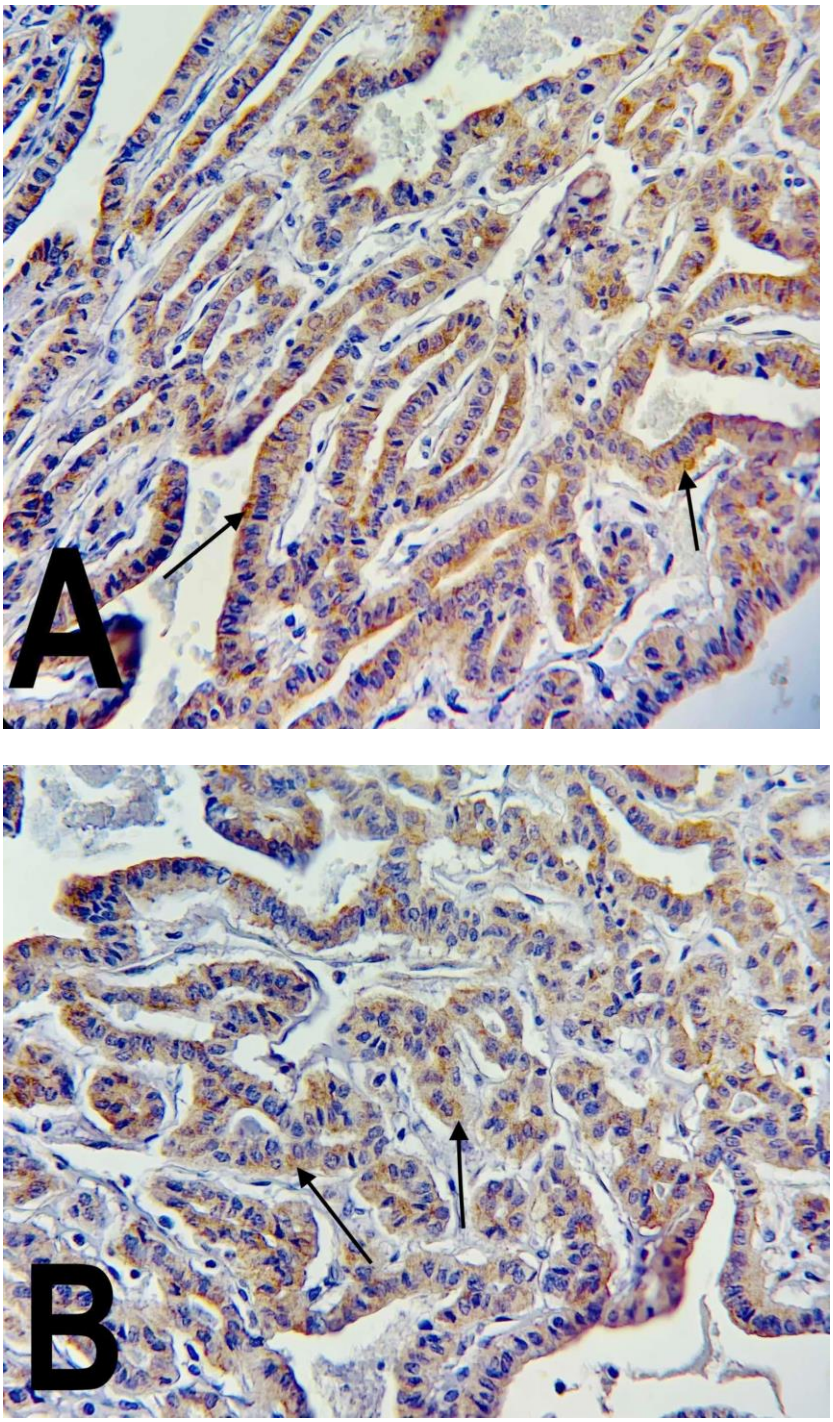


Figure (3): Papillary thyroid carcinoma positive moderate intensity cytoplasmic stain (arrows) for HPV 16 E6/ 18 E6 IHC. A 10x40. B 10x40

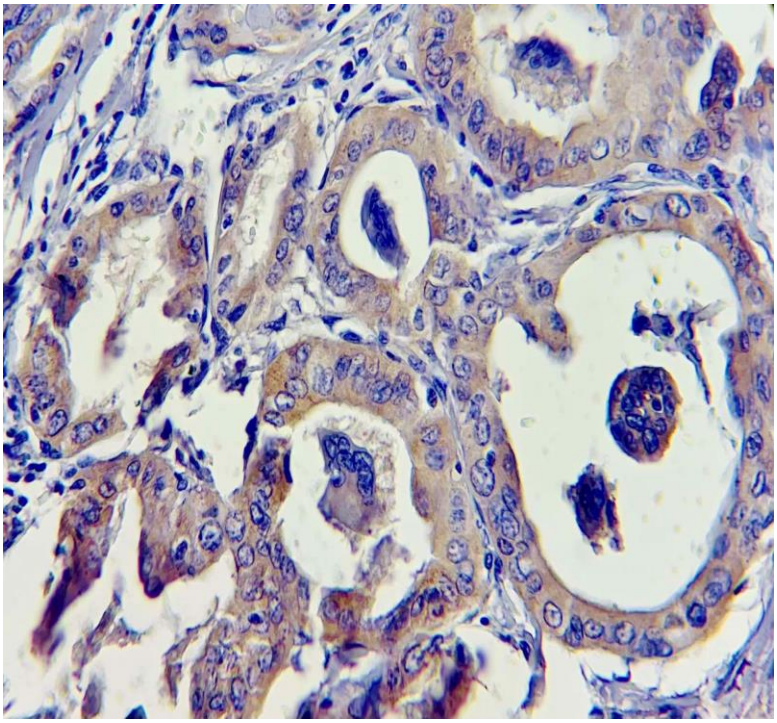


Figure (4): Papillary thyroid carcinoma positive moderate intensity cytoplasmic stain for HPV 16 E6/ 18 E6 IHC. 10x40

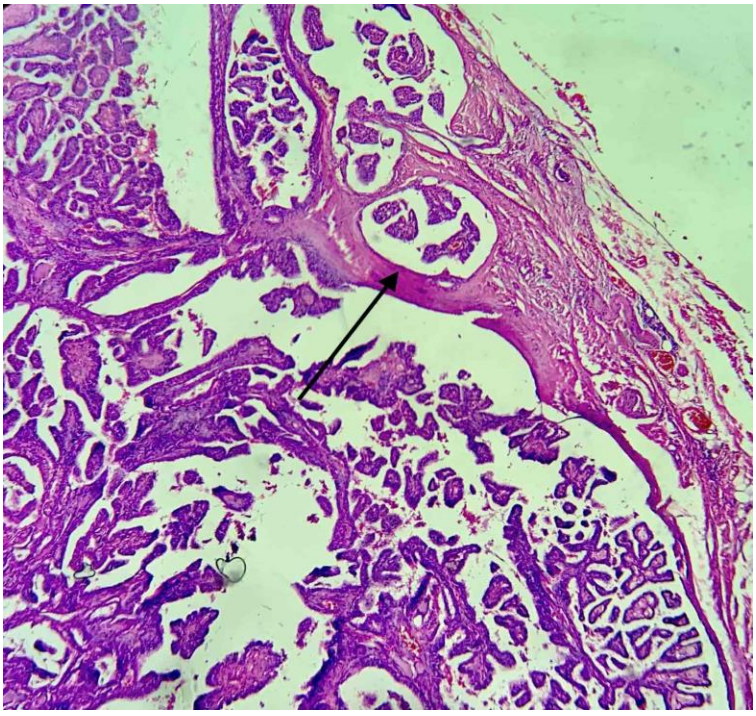


Figure (5): PTC complex true papillae with foci of capsular invasion(arrow). H&E 10*10

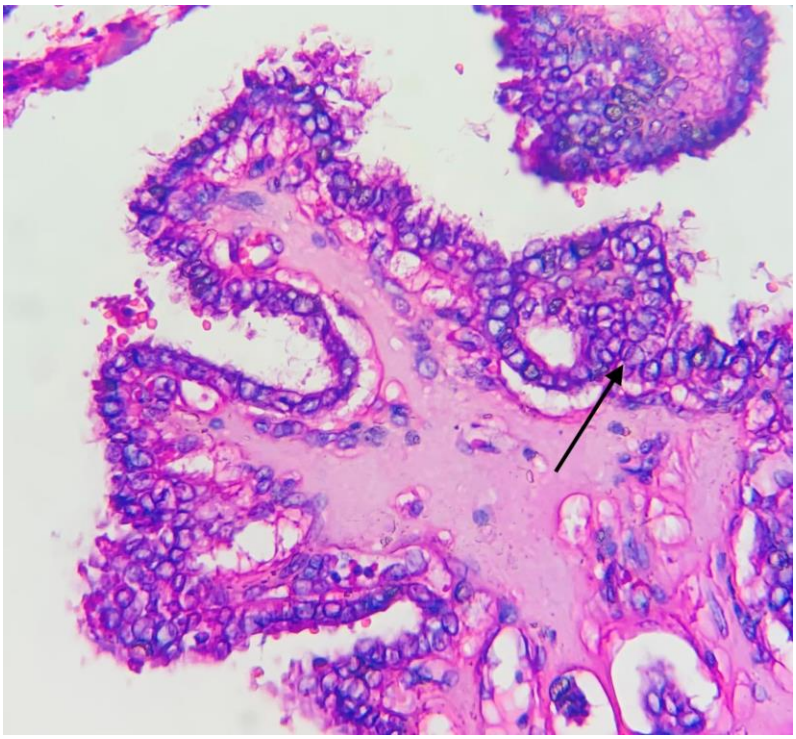


Figure (6): PTC with overlapped optically clear nuclei (Orphan Annie eye) and nuclear groove (arrow). H&E 10*40

Discussion:

People under 45 years of age were the predominant group in this study, the reason for dividing the cases into below and above 45 years of age is due to that PTC staging depends on dividing patients into these two groups [21].

This correlates with Bongarzone et al. study which indicates that in the incidence of RET and NTRK1 gene activation in papillary thyroid cancer is much greater in patients aged 4-30 years, supporting the idea that age may play a role in thyroid-specific carcinogenesis [23].

According to Fabio Muradas Girardi's study, the mean age of patients with PTC is 25-44 years [24]. The cause for such similarity may be due to few numbers of patient included in this current study. In this study 75.8%were females, and this is like many studies shows that women are roughly three times more likely than males to get papillary thyroid cancer [25]. Nevertheless, according to published standards, gender is not a risk factor in high-risk instances of papillary and follicular thyroid carcinomas [26].

According to Qui-square statistics where patients were classified into two age groups (above and below 45 years), neither age nor gender were significantly affecting HPV expression. however, when age was linked to HPV results using the independent sample t test using the mean age for all the patients studied rather than age groups, HPV expression in PTC sample was found to be significantly influenced by increasing age (mean age 49 ± 13.73560 SD).

A recent study conducted in April 2021 in Shiraz / Iran pointed out significant association between PTC and HPV positivity (13.4% positive) where the number of PTC samples studied were 82 sample [7].

There could be many reasons for such difference, small sample size in the current study is one possible reason, the fact that we used only histopathologically confirmed PTC samples (no benign samples were studied) and the type of HPV marker used (Anti-HPV 16/E6 and Anti-HPV 18/E6 Antibody).

A study in Mexico confirmed HPV positivity in 4.6% of patients with PTC as this study indicated that the presence of HPV is not frequent in thyroid neoplasms in general [(27)].

Statistically, we found no significance between HPV expression and PTC staging even though 4.5% of samples were positive for HPV and in stage I. that agrees with a study conducted in 2021 in Shiraz University of Medical Sciences, Shiraz, Iran which showed that There was no significant association between HPV positivity and tumour staging. [(7)]

Figure (4) above, shows that the IHC stain for HPV is cytoplasmic rather than nuclear. That can be explained in part by some reasons; firstly, we used Anti-HPV16 E6/ Anti-HPV 18 E6 Antibody (C1P5) which is according to a study conducted on cervical carcinoma [(28)] gives a cytoplasmic staining of the tumour cells with E6 oncoproteins and that is considered positive.

Second, while high-risk-HPV genome shown in cervical cancer is diagnosed in the great majority of instances and the HPV-DNA is still in episomal form, a small percentage of cervical malignancies develop [(29)]. An increase in episome copy number is detected in these cases, which is followed by an increase in the expression of viral oncogenes. That method achieves the same impact as HPV integration, i.e., high-level viral oncogene production leading to cell transformation, albeit less efficiently [(30)].

Moreover, Integration of the viral genome into the host one is shown in progressed stages of precancer and invasive cervical carcinomas, implying that HR-HPV genome assimilation occurs difficulty progressed in the aetiology of cancer [(31)]. Moreover, Inflammation-induced DNA damage typically occurs before HPV oncoprotein-induced genomic alterations [(32)]. As a result, HPV integration may play a role in neoplastic development. The current study has some limitations, including a shortage of potential research to prevent selection bias, a small sample size, and a lack of multivariate analysis.

In conclusion, there is a relation for human papillomavirus infection in development of papillary thyroid carcinoma (PTC).

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