RESEARCH ARTICLE

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Distribution of HPV Genotypes 16 and 18 among Resected Tonsillar Tissues from Pediatric Patients Operated for Non- Oncologic Nasopharyngeal and Palatine Tonsillar Hypertrophies

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ABSTRACT

Background: Recent advancements in molecular techniques have identified over 450 genotypes of Human Papillomavirus (HPV), classified into low- and high-oncogenic risk categories. The rise in high-oncogenic risk HPV genotypes has been linked to various cancers, including those affecting the oral, oropharyngeal, and nasopharyngeal regions in both pediatric and adult populations.

Methods: In this study, a cohort of 102 tonsillar tissue samples was included. This comprised 40 specimens from pediatric patients aged 4 to 9 years with nasopharyngeal adenoid hypertrophies, and 42 specimens from pediatric patients aged 5 to 12 years with palatine tonsillar hypertrophies. Among the 82 tonsillar tissue samples analyzed, 38 were from pediatric patients who underwent single-tonsillar type operations, while 22 were from pediatric patients who underwent dual-tonsillar type operations, resulting in a total of 44 tissues. Additionally, 20 control tissue samples were obtained from apparently healthy pediatric patients aged 5 to 12 years, following trimming operations of their inferior nasal turbinate tissues, which exhibited no notable pathological changes. For the detection of HPV 16/18 DNA, a recent iteration of Chromogenic in Situ Hybridization (CISH) technique employing specific DNA probes was utilized.

Results: In the analysis, among the 40 nasopharyngeal tonsillar hypertrophied tissues, 35.0% exhibited positive CISH reactions for HPV 16/18 DNA detection. Similarly, within the palatine tonsillar hypertrophied tissue group, 30.1% displayed positive CISH signals for HPV 16/18 DNA. For the 22 specimens obtained from dual-tonsillar type operations in the same pediatric patients (totaling 44 tissues), 45.5% showed positive-CISH signals for HPV 16/18 DNA at both sites. Notably, none of the control nasal tissues demonstrated positive-CISH reactions. Statistical analysis revealed a significant difference (P Value <0.05) when comparing the results of tonsillar hypertrophied tissues to those of the control group.

Conclusions: The notable presence of human papillomaviruses 16 and 18, particularly in their integrated forms of HPV-DNA, within pediatric groups exhibiting nasopharyngeal and palatine tonsillar non-oncologic hypertrophies, raises critical concerns regarding the potential spread of these high-oncogenic risk genotypes. These findings suggest that these sites may serve as reservoirs for the transmission of such viruses to adjacent mucosal tissues in the head and neck region. Furthermore, this presence of HPV could be a contributing factor in the pathogenesis, tumorigenesis, and carcinogenesis processes, constituting a significant step in this chain of events. Understanding these dynamics is crucial for developing effective strategies to prevent and manage the associated health risks in affected populations.

Keywords: Human Papilloma Virus 16/18, Chromogenic In Situ Hybridization, Palatine, Nasopharyngeal, Tonsillar, Hypertrophy, Tonsillectomy Adenoidectomy Adeno-Tonsillectomy.

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INTRODUCTION

Regarding the constitution of Waldeyer's ring, its anatomical definition declared that it comprises a group of lymphoid tissues, including lingual, palatine, tubal and pharyngeal tonsils (also termed as the adenoids)[1].

Tonsillar hypertrophy is an enlargement of tonsils (without tonsillitis) whereas adenoid hypertrophy is referred to the childhood condition of an increased size of adenoids and responsible for most common conditions affecting the adenoids which are associated with or without an acute or chronic adenoiditis[2]. The ICD-10-CM have listed the hypertrophy of adenoids, hypertrophies of tonsils with adenoids, and other cases with adenoid diseases and chronic tonsils, as J35.2, J35.3, and J35.8, respectively[3].

A list of infectious etiologies have been reported to infect nasopharyngeal and tonsillar tissues, among them many recognized viral agents, are irrelevantly to their altitude of existence including Coxsackie viruses, Parvovirus B19, coronaviruses, adenoviruses, Para influenza virus, Rhinovirus, herpes simplex virus, human Boca virus, human Epstein-Barr virus, human cytomegalovirus, and KI and KU Polyoma viruses[4-8].

Recently, advanced molecular techniques, and by using Next Generation Sequencing (NGS), have successfully recognized more than 450 human genotypes of papilloma viruses with ability to infect human skin tissues and epithelial surfaces of mucosae[9]. Around 25 types of Human Papillomavirus (HPV) are recognized for their high potential risk of oncogenicity, contributing to cancer development. Conversely, the remaining types are associated with low oncogenic risk, leading to non-malignant human lesions. Among these, HPV 6 and 11 specifically stand out. These are two most prevalent HPV genotypes that revealed to have an association with benign warts in anogenital area and papillomas of larynx[10]. The 'high- oncogenic risk' genotypes were found of having a causal link to the uterine cervical cancers while still the researchers have extending the causal relationships as well as associations to cancers that affecting other human anatomical sites, including cancers in the head and neck regions[11-13].

In recent years, there has been a notable rise in the detection rates of cancers associated with Human Papillomavirus (HPV) and Epstein-Barr virus (EBV) in the head and neck region. Specifically, the oropharyngeal region has seen a significant increase in HPV rates, with tonsillar tissues being particularly affected by these HPV infections[12-18].

Syrjanen (2004) on reviewing human papillomavirus infections in the nasopharyngeal tonsillar tissues had documented that the rates of documentation were poorly defined[19]. while Strzelczyk ET AL in 2022 and Kalantari ET AL in 2024 reported 60% and 58% prevalences of EBV infection in patients with chronic tonsillitis and tonsillar hypertrophy, respectively[18, 20].

The aim of the current research work aimed both to shed light on the rates of human papilloma viral genotypes 16 and 18 infections in the operatively resected tissues from non-oncologic cases of pharyngeal and palatine tonsillar hypertrophies from pediatric patients and to assess the physical DNA state of HPV 16/18 genotypes and their possible associative role in future tonsillar as well as oro- and naso-pharyngeal tumorigenesis/carcinogenesis.

MATERIALS & METHODS

Patients and Control: In this case-control prospective study, a total of 102 tissue specimens were gathered from the nasopharyngeal region of pediatric patients treated at the otolaryngology department in the Complex of Medical City in Baghdad, Iraq. Among these, 44 tissue specimens were obtained from 22 pediatric patients who underwent surgeries for both non-oncologic pharyngeal and palatine tonsillar hypertrophies. Additionally, 38 tissue samples were collected from pediatric patients who underwent surgery for either non-oncologic pharyngeal or palatine tonsillar hypertrophies individually. The remaining 20 tissue specimens were derived from pediatric patients whose apparently healthy nasal tissues, exhibiting non-remarkable histopathological changes following trimming operations for their inferior nasal turbinates, were included as a control group in the study. The detection of HPV 16/18 DNA was performed using the Chromogenic in Situ Hybridization (CISH) method. A recent version of the CISH kit was utilized, which included a digoxigenin-labeled oligonucleotide probe specifically designed to target HPV 16/18 DNA. This kit was procured from Zyto Vision GmbH, located at Fischkai, Bremerhaven, Germany, and the detailed instructions for performing the main steps of CISH procedures were done and followed accordingly to that stated by the manufacturing company.

At the histopathological laboratories of The College of Dentistry / Al- Mustaqbal University, each of the obtained tissues were processed for formalin-fixation and paraffin-embedding and then these blocks were subjected to serial cutting as thin sections of (4um) thickness and were stacked on single specified charged slide. We used only one cutting knife specified for each tissue block and then disposed so as to prevent from one tissue sample to another the possibility of carry-over DNA contaminations.

From each tissue block, the 1st couple tissue sections were specified for staining by hematoxyline and eosin whereas the followed many subsequent 4pm thickness-tissue sections were specified for the following procedures of in situ hybridization and tested for HPV 16/18-DNA.

These slides were incubated for 1 hr at 70°C and immersed twice times in absolute xylene (each for 5 minutes), then the slides had sequentially rehydrated at room temperature and had a drying step at 37°C for 5 minutes. Lastly, pepsin solution was applied for 45 minute at 37°C and kept in the distilled water, then after were air- dried.

In the initial step of Chromogenic in Situ Hybridization (CISH), a digoxigenin-labeled complementary

oligonucleotide probe specific for hybridization to the viral DNA of HPV 16/18 was applied to the slides. Subsequently, the slides were denatured at 75°C for 5 minutes, followed by hybridization at 37°C for 18 hours. The slides were then washed in (1x) TBS wash buffer at 55°C for 5 minutes.

Next, anti-digoxigenin-conjugated alkaline phosphatase-streptavidin was applied and incubated at 37°C for 30 minutes. The slides were washed in buffer TBS, followed by two washes in distilled water for 5 minutes each. They were then rinsed in detergent buffer for 5 minutes.

Following this, BCIP/NBT was applied and incubated at 37°C for 30 minutes until a dark-blue precipitate developed at any positive sites in the examined tissues. The slides were counter-stained with Nuclear Fast Red (NFR), and the sections were dehydrated sequentially with ethyl alcohol and mounted with Disteren Plasticizer Xylene.

Assessment of the CISH signaling results of HPV 16/18-DNA detection: The development of CISH staining signals at nuclear localization are considered as a positive results of HPV 16/18-DNA detection. These were elicited as an intense blue -colored signals of the precipitates which were detected at the positive complimentary sites of the probes to their specific HPV 16/18-DNA in the examined cells and were assessed in 10 different high power fields. The CISH signal patterns were categorized into two types: Diffuse (D) and Punctuated (P). The Diffuse Pattern (D) was characterized by nuclei that were completely stained. In contrast, the punctuated Pattern (P) was identified by the presence of distinct intranuclear dot-like signals. The mixed physical pattern were representing both the collective Diffuse and Punctuated (D/P) physical patterns[21].

The light microscopy was being used for counting the positive cells at X400 by the quantification of the in situ hybridization signals, and were specified as to have both intensity of positive signal of reactions (as low, moderate, and high intensity scores) and specified as to have the scores results that was determined basing on the average number of cells showing positive signals and counted in 10 different fields of 100 cells of each examined tissue sample and then assigned to have score(1) category when cells showing positive signals revealed in 1- 25% of examined cells while score(2) when cells showing positive signals revealed in 26-50%, and Score(3) when cells showing positive signals revealed in >50% of examined cells[22].

Statistical analysis was conducted using SPSS-23 package to calculate the significance (p value). The Chi-square test was employed to determine the statistical significance of the results concerning the studied parameters. This analysis examined the relationships between various variables, with a p value less than 0.05 indicating a significant relationship between the parameters under study.

RESULTS

Pediatric Patients with Nasopharyngeal and Palatine Tonsillar Hypertrophies as Age- Distributed Results: The tissue specimens collected in this study were obtained from pediatric patients who underwent surgery for nasopharyngeal and palatine tonsillar hypertrophies, with ages ranging from 4 to 12 years. The mean age for patients with nasopharyngeal hypertrophies was (5.77 ± 3.73) years, and for those with palatine tonsillar hypertrophies, it was (6.33 ± 3.44) years. Additionally, the mean age of apparently healthy individuals (A.H. control) was 6.35 ± 5.66 years, with an age range of 5-12 years. Statistical analysis revealed non-significant differences ($P>0.05$) among the studied groups (**Table 1**).

Sex distribution of pediatric patients with nasopharyngeal and palatine tonsillar hypertrophies: Males with nasopharyngeal tonsillar hypertrophies were higher (60%: 24) than their female counterparts (40%: 16). In addition, pediatric males with palatine tonsillar hypertrophy were higher (59.5%: 25) than their female counter parts (40.5%: 17). Also, in the control group, the males were higher (60%: 12) than their female counterparts (40%: 8). The statistical analysis showed highly significant differences ($P<0.01$) among the studied groups in relation to their gender (**Table 2**).

CISH Expression of Human Papilloma Viral Types 16/18 DNA in Nasopharyngeal and Palatine Tonsillar Hypertrophied Tissues

I-Signal scoring of positive HPV 16 / 18 DNA- CISH reactions: The nasopharyngeal tonsillar hypertrophied tissues exhibited positive signals for HPV 16/18 DNA in 35% of cases, representing 14 out of 40 tissues in this group. Signal scoring revealed 15% with low signals, 12.5% with moderate signals, and 7.5% with high signals (detailed in **Table 3** & **Figures 1-4**). Similarly, the palatine tonsillar hypertrophied tissues displayed positive signals in 30.1% of cases, representing 16 out of 42 tissues. Signal scoring revealed 14.3% with low signals, 16.7% with moderate signals, and 7.1% with high signals, (**Table 3**). None of the control tissue group presented positive signals for HPV 16/18-CISH test. However, when comparing the percentage of HPV 16/18 DNA scoring among nasopharyngeal tonsillar hypertrophied tissues, palatine tonsillar hypertrophied tissues, and AHC groups, the differences were statistically highly significant ($P<0.01$).

II-Assessing signal intensities of CISH reactions for HPV 16/18 DNA: Regarding signal intensities of HPV 16/18-CISH signal detection in nasopharyngeal tonsillar hypertrophied tissues, weak signal intensity was observed in 20%, while 10% and 5% of these tissues exhibited moderate and strong intensities, respectively. Statistically significant differences were recorded between the studied groups at ($P<0.01$) (**Table 4 & Figure 1**).

Regarding signal intensities of HPV 16/18-CISH signal detection in palatine tonsillar hypertrophied tissue group,

Table 1: The studied pediatric patients with nasopharyngeal and palatine tonsillar hypertrophies as distributed according to their age results.

Studied groups (Age / Year)	N	Mean	Std. Deviation	Std. Error	Range		ANOVA test (P- value)
					Minimum	Maximum	
Nasopharyngeal Tonsillar Hypertrophies	40	5.77	3.73	1.11	4	9	
Palatine Tonsillar Hypertrophies	42	6.33	3.44	1.21	5	12	P=0.4 (P>0.05)
A.H. Control	20	6.35	5.66	2.14	5	12	
Total	102						

Table 2: Distribution of the studied pediatric patients with nasopharyngeal and palatine tonsillar hypertrophies according to their gender.

Gender		Studied Groups			Pearson Chi-Square (P-value)
		Apparently Healthy Control	Nasopharyngeal Tonsillar Hypertrophies	Palatine Tonsillar Hypertrophies	
Male	N	12	24	25	P=0.005
	%	60%	60%	59.5	
Female	N	8	16	17	Sign (P<0.01)
	%	40%	40%	40.5	
Total	N	20	40	42	

Table 3: Distribution of signal scores of HPV 16 / 18-DNA-CISH reactions among the studied nasopharyngeal and palatine tonsillar hypertrophied tissue groups.

HPV 16 / 18 CISH reaction scores		Studied groups			P-Value
		A.H. Control	Nasopharyngeal Tonsillar Hypertrophy	Palatine Tonsillar Hypertrophy	
Negative	N	20	26	26	
	%	100%	65.00%	61.90%	
Positive	N	0	14	16	
	%	0.00%	35.00%	30.10%	
Low	N	0	6	6	
	%	0.00%	15.00%	14.30%	
Moderate	N	0	5	7	P=0.000
	%	0.00%	12.50%	16.70%	Highly
High	N	0	3	3	Sign. (P<0.01)
	%	0.00%	7.50%	7.10%	
Total	N	20	40	42	
	%	100%	100%	100%	

weak signal intensity was observed in 21.4%, while 11.9% and 4.7% of these tissues had moderate and strong intensities, respectively. Statistically significant differences were recorded between the studied groups at (P<0.01) as detailed in (**Table 4 & Figure 1**).

III-Physical state assessment of HPV-16/18 DNA as an integrated and episomal form in the examined tissues

Regarding episomal and integrated forms of HPV 16 / 18-CISH signal detection in nasopharyngeal tonsillar hypertrophied tissues group, the episomal was noticed in 28.6 % whereas 71.4% have an integrated forms. Assessing the physical state of HPV-16/18-DNA in pallatine tonsillar hypertrophied tissues group revealed the episomal was noticed in 56.3 % whereas 43.7% of the examined tissues having an integrated forms (**Table 5**).

IV-Results of CISH expression of human papilloma viral types 16 / 18 DNA in tissues from pediatric patients sustained combined nasopharyngeal - palatine tonsillectomies: The results of CISH expression of HPV 16/18 among 22 combined tissues from pediatric patients who underwent combined nasopharyngeal-palatine tonsillectomies for their tonsillar hypertrophies revealed 45.5% positive signals for HPV 16/18-CISH test, representing 10 out of the examined 22 tissues in this group. A statistically significant difference was recorded between the studied groups at (P<0.05) (**Table 6**).

V-Spearman's Rho statistical testing to evaluate the studied markers in hypertrophied nasopharyngeal and palatine tonsillar tissues. A strong positive relationship (with highly significant correlation) was found between HPV 16 / 18 and age of pediatric patients with nasopharyngeal and palatine tonsillar hypertrophies (r = 0.232, P = 0.004). However, no significant correlation between HPV 16 / 18 and gender of the examined pediatric patients (**Table 7**).

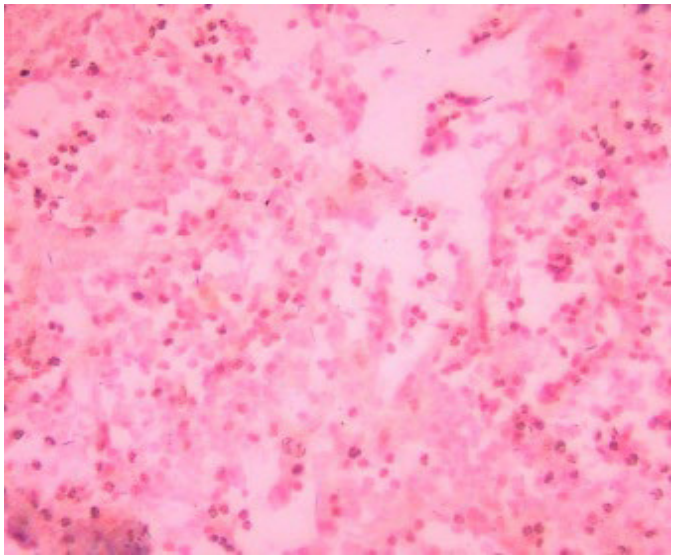

Figure 1: Hypertrophied nasopharyngeal tonsillar tissue shows no HPV 16/18-DNA -CISH reaction (20X).

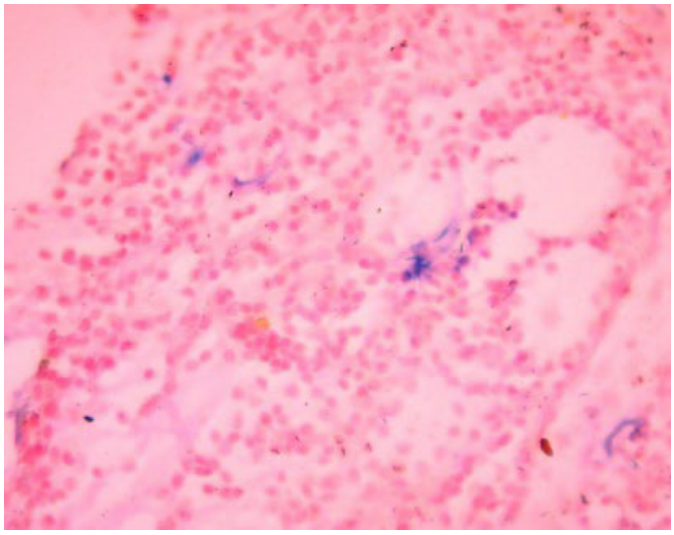

Figure 2: Hypertrophied palatine tonsillar tissue with positive HPV 16/18-DNA -CISH reaction (20X).

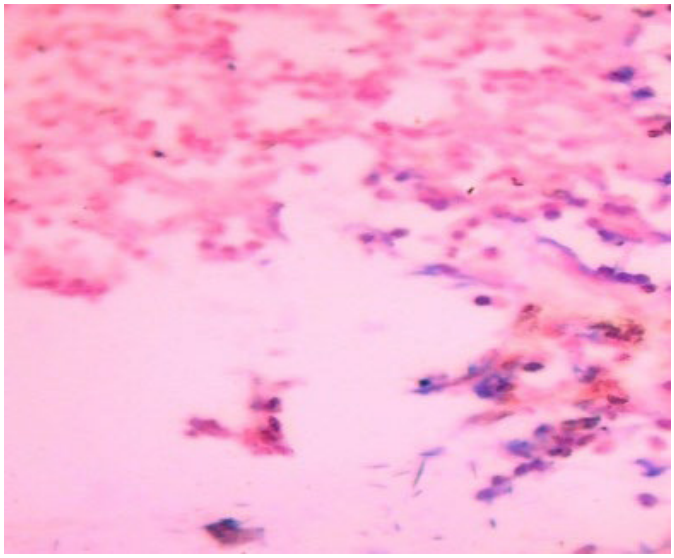

Figure 3: Palantine tonsillar tissue shows moderate score and moderate intensity scoring grades of positive HPV 16/18-DNA-CISH-reaction (40X).

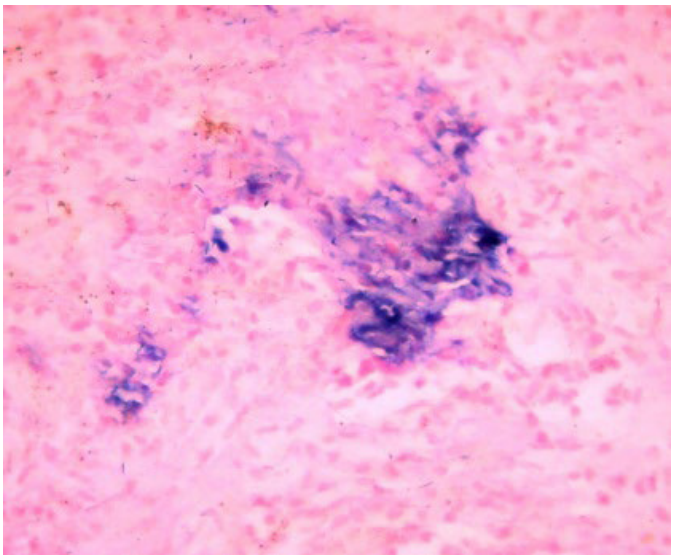

Figure 4: Nasopharyngeal tonsillar tissues shows high score and strong intensity scoring grades of positive HPV 16/18-DNA -CISH reaction (40X).

Table 4: Distribution of signal intensities of HPV 16 / 18-DNA-CISH reactions among studied tonsillar hypertrophied tissue groups.

HPV 16 / 18 intensity		A.H. Control	Studied groups		P-Value
			Nasopharyngeal Tonsillar Hypertrophy	Pallatine Tonsillar Hypertrophy	
Negative	N	20	26	26	P=0.004 Highly
	%	100%	65%	61.90%	
Positive	N	0	14	16	
	%	0.00%	35%	30.10%	
Weak	N	0	8	9	
	%	0.00%	20%	21.40%	
Moderate	N	0	4	5	
	%	0.00%	10%	11.90%	
Strong	N	0	2	2	
	%	0.00%	5%	4.70%	
Total	N	20	40	42	
	%	100%	100%	100%	

Table 5: Physical state assessment of HPV-16/18 DNA in the examined tonsillar hypertrophied tissues.

Physical state of HPV-16/18 DNA in the examined tissues	Nasopharyngeal Tonsillar Hypertrophy		Pallatine Tronsillar Hypertrophy	
	Positive (No.)	%	Positive(No.)	%
Episomal form	4	28.6	9	56
Integrated form	10	71.4	7	44
Total	14	100	16	100

Table 6: Results of CISH expression of HPV 16 / 18 DNA in tissues from pediatric patients with combined nasopharyngeal- palatine tonsillectomy operations.

CISH expression of HPV 16 / 18 DNA	Pediatric patients with combined nasopharyngeal- palatine tonsillectomy operations (No. = 22)	
	N	%
Negative*	12	54.5
Positive*	10	45.5
p.value	Sign. (P<0.05)	

* The expression of positive and negative results of CISH reactions for HPV 16 / 18 DNA incidentally found or missed, respectively, in both nasopharyngeal- palatine tonsillectomied tissues while none of them revealed expression of positive and negative results of CISH reactions in nasopharyngeal and palatine tonsillectomied tissues individually.

Table 7: Spearman's Rho statistical testing of age, gender, and HPV 16\18-CISH to evaluate the studied markers in nasopharyngeal adenotonsillar tissues.

Spearman's rho		Age groups (years)	Gender	HPV 16/18
Age groups (years)	r		0.04	
	P		0.873	
Gender	r			0.174
	P			0.355
HPV16 / 18	r	0.232		
	P	0.004*		

*Correlation is highly significant (P<0.01)

DISCUSSION

Burley et al, and at early 2020, reported that the Papillomaviridae as a family have included over 450 distinct human HPV types[23]. HPVs have revealed an evidenced role in cervical cancer, head and neck squamous cell carcinoma, esophageal cancers and brain as well as lung tumors[24]. The first who proposed the link between HPV and oral squamous cell cancer was Syrjanen et al., in 1983[25]. Then the HPV rates in relation to head and neck cancers in the previous researches have been increased, where the most commonly affected were the tonsillar tissues, however, the current rates of HPV infection in the pediatric populations are still poorly determined[12]. In this respect, only scarce researches which had studied HPV DNA, in only 200 biopsies, with tonsillitis and normal tonsillar tissues and up to the year 2003, where a 8.5% and out of them contained DNA of this virus[19].

It is clear that the same high-risk HPV types, mostly HPV16 and HPV18, are predominantly found in both cervical cancer and oral cancers, and further, a theory regarding the importance of high -risk HPV genotypes, among many other viral infections, via similar mechanisms in their ability for immortalization and transformation of both human cervical epithelia, oral keratinocytes and upper aero- digestive tract epithelia has been raised[26, 27].

It's great to acknowledge the limitations in the research, this study and as far as we are aware, is representing the first pioneered research of its kind in our country (Iraq), that have rating the HPV 16 and 18 infections, by using a recent version of in situ hybridization technique, in pediatric patients underwent surgical procedures for non-oncologic hypertrophy of the nasopharyngeal and palatine tonsils.

In view of these facts and speculations, the aim of this research was to evaluate the percentage of HPV genotype 16/18 infections, by using Chromogenic in Situ Hybridization Analysis (CISH), in pediatric patients who have undergone either an individual routine tonsillectomy and an individual routine adenoidectomy or those pediatric patients who undergone combined operations (by tonsillectomy as well as adenoidectomy) for their non-oncologic hypertrophy of the nasopharyngeal and palatine tonsils.

The present study have enrolled a total of one hundred and two (102) tissue blocks from pediatric patients who have undergone tonsillectomies and / or adenoidectomies for CISH detection of HPV genotype 16/18 in the enrolled tonsillar tissue specimens.

In this research work, statistically significant differences (p-value < 0.01) among the group of palatine tonsillar hypertrophied tissues that revealed 30.1% positive CISH test signals of HPV genotype 16/18 (16 out of 42 tissues) as well as among the group of the nasopharyngeal tonsillar hypertrophied tissues that revealed 35.0% positive CISH test signals of HPV genotype 16/18 (14 out of 40 tissues) in comparison to the control tissues that revealed no positive-CISH test results of HPV genotype 16/18.

However, two previous Iraqi researches by Ali et al.[28] in 2011 and Ali et al.[29] in 2017 have revealed positive- CISH signals of HPV DNA in 3.2% as well as 10%of those healthy appeared on histopathological examination and used as oral control tissues and healthy nasal control tissues in Iraq. Furthermore, previous Iraqi study by Ali et al in 2016[30] and by using ISH had also found multiple/ mixed HPV infections in Iraqi patients with laryngeal carcinoma.

In addition, a systematic review by Wojtera et al.(2018)[12] revealed a rate of HPV that was ranging from 0 to 21% in tonsillar tissues resected from pediatric patients. However, a previous study[31] (who enrolled 1670 patients) as well as their four studies which were reviewed by them (consisting of 1941 patients) have not detect HPV in these tissue specimens. In other studies[32-33] that enrolled a largest patient samples, only 1% positive results have been reported, that was relating these results for the potentialities of sample or selection bias. In most previous studies, the broad-spectrum HPV primers used in a conventional PCR method (which is still unreliable) have yielded high false positive rates[34]. However, the gold standard method of RT-qPCR was used to demonstrate the altitude of HPV transcriptional expression[35].

Moreover, Wojtera et al. in 2018[12] reviewed seven previous studies, that had evaluated HPV infection in a number of tissues (via tonsillectomies) from pediatric patients with tonsillar hypertrophy and chronic tonsillitis, ranging from 8-1,670, and they reported an overall rate ranging from 0 to 21%, and since were previously revealed as conditions which were not well-related to HPV infection, they might be possibly assessed as confounders[14].

Many of the following possibilities were suggested for the reported high HPV prevalences as well as differences in the rates in the previous researches as lacking controls, differences related to the HPV subtypes, and variable methods for testing, and because most of them still had tested by unreliable conventional PCR that utilized primers having poor specificity and associated with high false positivity rates[15].

On the other hand, a previous study[31] had assessed 44 types of HPV by using PCR technique with an enzyme immunoassay facility for a number of 3,377 FFPE specimens and from 511 homogenized tonsils, found no HPV DNA as well as no evidence for premalignant neoplasia with an association with HPV in all of the tested samples and had concluded that HPV infection may be rare in tonsillar epithelia.

Either physical form (the integrated and episomal expressed forms) of HPV 16/18 DNA detection were seen as CISH signal in these currently examined nasopharyngeal as well as palatine tonsillar hypertrophied tissues. Importantly, the nasopharyngeal tonsillar hypertrophied tissues have revealed an integrated physical pattern in 71.4% (10 out of 14 tissues) of the total positive CISH test signals of HPV genotype 16/18 of this group of tissues while in their counterpart group of palatine tonsillar hypertrophied tissues have revealed an integrated physical pattern in 43.7% (7 out of 16 tissues) of the total positive HPV genotype 16/18 of this group of tissues. Regarding HPV physical state of HPV-DNA in tonsillar carcinomas, only a few previous studies had systematically analyzed this respect, where both an episomal (diffused) and an integrated (punctuated) forms have been reported[12, 21, 13, 36-40].

CONCLUSION

It seems crucial to evaluate the rates of HPV infections and their typing in such pediatric population. However, it seems also such difficult issue when aiming to unravel whether these HPV 16 /18 infections in the studied pediatric tonsillar tissues represent just a transient infections that eliminated eventually by the immune system of the affected children. It is also of importance to know when have first acquired, and to delineate the periods of such HPV 16/18 latently viral status in these tonsillar tissues and will remains as such status.

Additionally, it is currently unknown and is worth to note and to be determined, too whether these HPV 16/18 infections would have an eventual progression to symptomatic disease or a possibility of later development into a pre-malignant states which ultimately lead to a malignant lesions.

Although previous data had suggested that HPV-associated premalignant lesions do rarely occurred, but since these HPV 16/18 genotypes are proved to have high oncogenic potentialities, the tissues have these viruses are likely to have a high risk to progress into cancerous states.

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