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Detection of human papilloma virus (HPV) in tonsils of children undergoing tonsillectomy at Muhimbili national hospital

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Abstract

Background: The Human papillomavirus (HPV) related tonsillar carcinoma is skyrocketing worldwide. It affects people at younger age usually without prior history of alcohol and tobacco use. The persistence of high risk HPV tonsillar infection in early in childhood may progress to tonsillar carcinoma. Parent HPV risk factors increases the chances of HPV vertical transmission in children. Given high prevalence of oropharyngeal carcinoma in Tanzania, little is known about the prevalence of HPV in tonsils of children and associated maternal HPV risk factors. This study aimed at detecting high risk HPV in tonsils of children undergoing tonsillectomy.

Materials and Methods: A descriptive hospital-based cross sectional study was carried out from December, 2020 to May, 2021 at the Departments of Otorhinolaryngology and Pathology at Muhimbili National Hospital, and Ifakara Health Institute laboratory at Bagamoyo. It included admitted patients aged 2-12 years old scheduled for routine tonsillectomy, and their mothers who completed a questionnaire designed to assess risk factors to HPV infection. The tonsillar specimens were collected, fixed, then HPV Deoxyribonucleic acid extracted, amplified and detected using Anyplex HPV assays. Data were entered on SPSS 23 and Fischer exact test was used to assess association.

Results: Out of 50 tonsillar specimen, the HPV DNA were detected in 4 (8%). The relationship of maternal risk factors; education level, age at delivery, number of sexual partners and mode of delivery was not statistically significant with HPV positive tonsils in children

Conclusion: The 8% proportion of HPV DNA pediatric tonsils signifies that there is detectable high risk HPV in pediatric population that needs further larger and more powered studies.

Keywords: Human papilloma virus; Tonsils; Tonsillectomy; Children; Tanzania.

1. Introduction

There is an increasingly growing body of knowledge linking human papilloma virus particularly the high risk type, to development of tonsillar carcinoma(1,2). The tonsillar carcinoma represents about 55% of all oropharyngeal cancer. The prevalence of HPV related tonsillar cancer in different parts of the world estimated to be around 70% (3,4). To date, there are more than 100 genotypes of HPV DNA recognized, 30 HPV genotypes of which 15 are high risk, are involved in causing disease to humans in different epithelia of the body(5).The HPV 16 genotype is by far the most isolated DNA from the tonsillar carcinoma in up to 90% of cases(6).

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The HPV oncogenes E6 and E7 degrade and inactivate tumor suppressor genes P53 and Rb respectively; this in turn leads to chromosomal instability and accumulation of genetic mutations and ultimately development of premalignant and malignant lesions(7). The HPV tonsillar infection is mostly acquired in childhood and may progress to tonsillar carcinoma in adulthood if the infection persists more than 20 years. It is not known why some people are able to clear the infection within 2 years while others cannot (8). The HPV transmission rate from the mother to her newborn ranges from 4% to 72 %(9). A fetus can get infected during the time of conception from an infected sperm or ovum, in utero through the placenta and during giving birth when fetus passes through an infected birth canal(10).

Despite the fact that a few number of studies have been done on the detection of HPV in tonsils of children worldwide, most of these studies were done in developed countries in Europe, North America and Asia. Little has been done in Africa where the risk factors for the development of HPV related tonsillar carcinoma is almost the same. Moreover, the prevalence of oropharyngeal cancer is high but there is no study that has been done on its association with HPV.

Furthermore, some previously done studies included subjects who were vaccinated against HPV or did not declare the vaccination status of their subjects (8,11).This might have had an impact to the results of the study as part of the studied population would already have immunity against HPV. The chances of not finding HPV DNA from this kind of samples were high. This study did not involve subjects with prior history of vaccination against HPV so as obtain the real picture of the studied population. This study aimed to specifically determine the proportion, genotypes of high risk HPV in tonsils of children and assess maternal HPV risk factors and HPV positive tonsils in children

2. Material and method

2.1. Study design

This was hospital based descriptive cross sectional study

2.2. Study duration

The study was done for six months from December, 2020 to May, 2021

2.3. Study site

The study was conducted in Otorhinolaryngology (ORL) and Pathology departments at Muhimbili National Hospital (MNH) and Ifakara Health Institute (IHI) laboratory at Bagamoyo. The MNH is also a teaching hospital and a research centre for the Muhimbili University of Health and Allied sciences. The hospital has a total of 25 specialized departments including ORL. The ORL department performs on average 30 tonsillectomies per month in the pediatric population, with common indications being Obstructive tonsillitis, Chronic and Recurrent tonsillitis. The pathology department on average receives 40 biopsy specimens per day from hematology and all surgical departments including ORL. The IHI laboratory at Bagamoyo is one among ISO accredited laboratories in the country. It's well equipped with the basic and state of the art equipment for molecular biology with highly skilled and experienced laboratory scientists

2.4. Study population

The study involved children aged 2-12 year admitted in MNH-ORL department with non-malignant tonsillar conditions, and their mothers who completed a questionnaire that was assessing the risk factors to HPV infection.

2.5. Sample size estimation

The sample size of this study was estimated on the basis of Fischer's Formula and from the previous study by (12)
Sample size calculations:

$$N = \frac{Z2P\ (1-P)}{d^2}$$

Where;-

N= estimated sample size, Z= the standard normal deviates which is 1.645 using the 90% confidence interval, d=Margin of error 0.1 and P=Previous Prevalence of HPV DNA, 12.5%

$$N = \frac{1.645\text{^}2 * 0.125(1-0.125)}{0.1\text{^}2}$$

= 30

Adjusting for non-responders by taking into account an approximate of 10% of the sample size with no response which gives a response rate (R) of 90%. Therefore, adjusted sample size N×1/R= 33. However, our study included 50 specimen from children undergoing tonsillectomy.

2.6. Sample selection

Convenience sampling was done to obtain samples in this study from children aged 2-12 years admitted in MNH-ORL department with non-malignant tonsillar conditions scheduled for routine tonsillectomy and whose mothers were available. The study excluded children with history suggestive of tonsillar malignancy, vaccination against HPV and hose mothers were unavailable.

2.7. Sample and Data Collection

Recruitment of patients was done in ORL wards for those children scheduled for tonsillectomy due to obstructive tonsillitis, recurrent or chronic tonsillitis. After tonsillectomy, the right and left tonsils were collected in a formalin container and transported to histology laboratory where formalin fixation and paraffin embedding (FFPE) was done. Having done all the FFPE specimens, they were transported by the principal investigator to IHI laboratory where the DNA extraction and detection was carried out. The extraction and detection of HPV genotypes was done by a molecular biologist trained on real time PCR and Anyplex HPV detection assays.

The Anyplex II HR HPV detection is a multiplex real-time PCR assay that permits the simultaneous amplification, detection and differentiation of target nucleic acids of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) as well as Internal Control (IC) to ensure validity of the findings. The assay uses Human house-keeping gene as an endogenous IC which can ensure purification of DNA, verification of PCR reaction and clarification of cell adequacy from each specimen. In summary, a master mix was prepared containing primers, DNA polymerase, dNTPs and RNase free water. Then 15 microliter of the master mix was aliquoted into the PCR tubes followed by adding 5 microlitre of the sample on each tube. The mixture was then run based on CFX96TM Real-time PCR System (Bio-Rad) protocol for detection of 14 HR HPV. Then, results were interpreted in accordance with the fluorophores that were detected on specific fluorescent wells.

Thereafter, the mothers of the children whose specimen were collected, completed the questionnaires designed to assess risk factors for HPV infection such as age at delivery, education level of the mother, mode of delivery and number of sexual partners.

2.8. Data Analysis

Data entered on statistical software SPSS version 23. Frequency distribution tables used to summarize data. The association of risk factors was done using Fischer exact test at confidence of 95% and p- value < 0.05

3. Results

3.1. Socio demographic characteristics of study participants

The tonsillar specimen was obtained from 50 children, male and females were in equal number. The mean age of participants was 4.2 years ranging from 2-11 years with majority in the age group 6 - 9 years. The mothers' participants were also 50 in number; about half of them were in the age group 31- 40 years. Most of the mothers (40%) had secondary education as shown in table 1.

Table 1 Socio demographic characteristics of study participants

A: Children	**Frequency**	**n %**
Age group (years)		
2 - 5	14	28
6 - 9	25	50
>9	11	22

Sex		
Female	25	50
Male	25	50
B: Mothers		
Age groups (years)		
< 21	1	2
21 - 30	17	34
31 – 40	26	52
41 - 50	6	12
Education level		
No formal education	1	2
Primary	14	28
Secondary	20	40
College/University	15 50	30 100

3.2. Proportion of high risk HPV tonsils in children

The proportion of HPV positive tonsils in children was 8% as shown in table 2. The proportion of children with HPV positive tonsils was higher in the age group 2-5 year when compared to other age groups. The difference was not statistically significant (P-0.7)

Table 2 Proportion of high risk HPV tonsils in children

		HPV positive	**HPV negative**	**Total**	**P- Value**
SEX	Males	3(12)	22(88)	25(50)	
	Females	1(4)	24(96)	25(50)	
Total		4(8)	46(92)	50(100)	
AGE (years)	2-5	2(14.3)	12(85.7)	14(28)	0.7
	6-9	2(8)	23(92)	25(50)	
	>11	0	11(100)	11(22)	
Total		4(8)	46(92)	50(100)	

3.3. Detection of HPV DNA genotypes

Among the detected HPV DNA genotypes, HPV DNA 16 (75%) was the most common genotype. HPV DNA 18 represented the remaining (25%).There was no other high risk HPV DNA genotype detected as shown below.

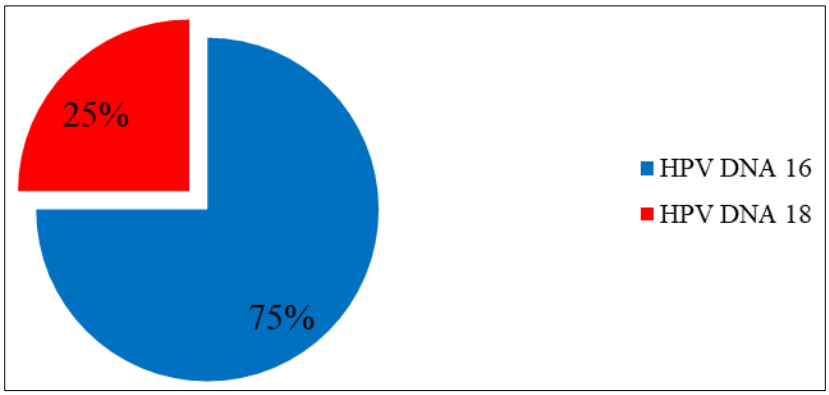

Figure 1 Detection of HPV DNA genotypes

The proportion of children with HPV positive tonsils was higher among mothers with primary education when compared to mothers with secondary and College/University education.(P = 0.7)

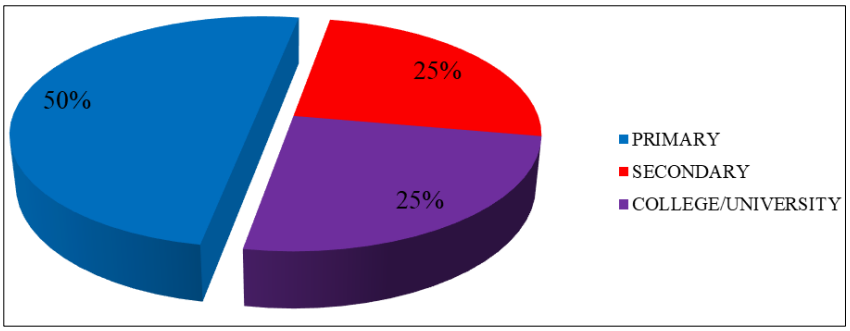

Figure 2 Level of education and HPV positive tonsils in children

The proportion of children with HPV tonsils was higher among mothers who had more than one sexual than those with one sexual partner. (P = 0.69)

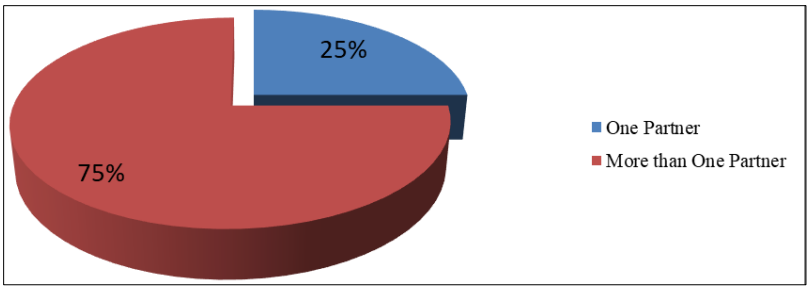

Figure 3 Number of maternal sexual partners and HPV positive tonsil in children

The proportion of children with HPV positive tonsils was higher among mothers who had delivered in the age group of 21-30 years when compared to other age groups. (P = 0.38)

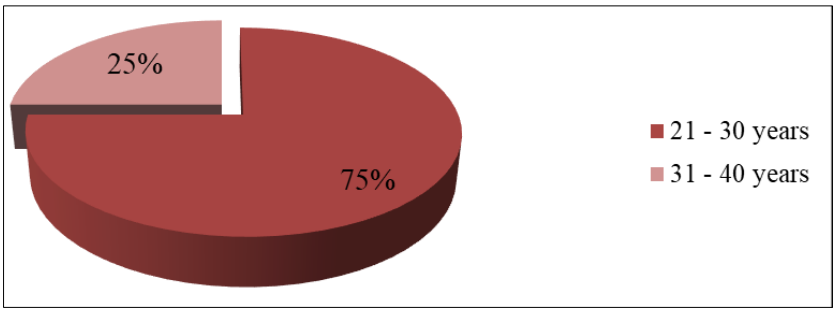

Figure 4 Maternal age at delivery and HPV positive tonsils in children

The proportion of children with HPV positive tonsils was higher among mothers who delivered via normal vaginal delivery than those who delivered by C-section. (P = 0.65)

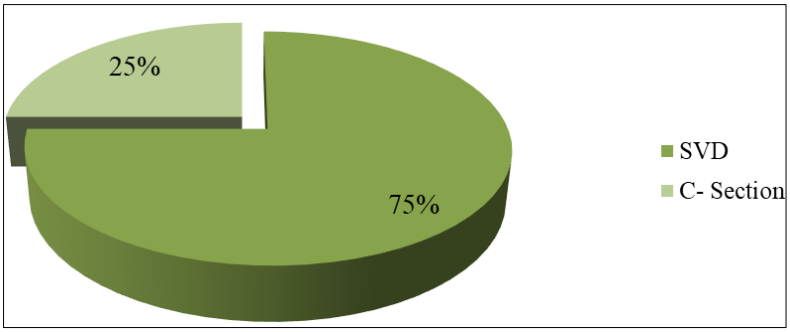

Figure 5 Mode of delivery and HPV positive tonsils in children

4. Discussion

In the view of increase in incidence of HPV related tonsillar carcinoma, it's imperative to study HPV DNA in normal tissues to understand more of its epidemiology and pathogenesis. In this study, we demonstrated that the prevalence of HPV positive tonsils in children who underwent tonsillectomy was 8%. Among children with HPV positive tonsils, the age group of 2-5 years had a higher prevalence than other age groups. These findings are in keeping with those of Mammas et at(13) whose prevalence in HPV DNA from tonsillar tissues was 9.4% and preschool more common affected and was statistically significant. In a similar study by Chen et al(14) also showed consisted findings of HPV DNA prevalence 8.5% and common in the preschool as above. However, there were various studies with even large sample size that reported zero prevalence of HPV DNA from tonsillar specimen. E. Pamer et at(15) from the UK studied 4095 samples from tonsillar specimen in both fresh tissue and paraffin embedded reported zero prevalence. This was also consistent with a study from Brazil(16) with a sizeable sample size. In the present study, we detected HPV DNA in four tonsillar specimens by using Anyplex II high risk HPV detection assays. The detected genotypes were HPV DNA 16 and 18 and the former was the most common in 75% of cases. These findings were in agreement with various other studies including a study that was carried out in China and Pakistan(6) that detected HPV 16 DNA was only high risk genotype from positive tonsillar samples.

This study has also demonstrated that among mother of children with HPV positive tonsils, higher prevalence was seen in mothers, who had lower education level, more than sexual partners and those in the age group of 21-30 years. Smith et al (17) in the study of evidence of for vertical transmission of HPV from mothers to infants revealed that mothers with lower education were associated with increased risk of maternal HPV DNA. This was also in keeping with findings of Renato et al(18) that showed illiterate women had a higher prevalence (58%) of HPV DNA than women with other levels of education. In the present study, it was shown that among children with positive HPV tonsils those born through normal vaginal delivery (SVD) had a higher prevalence of HPV positive DNA than those born through C-section. This finding was consistent with the findings of the Meta analysis on the effect of mode of delivery on maternal HPV transmission that showed C-section was associated significantly in reduction of HPV transmission than vaginal delivery(19)

However, a study done previously by IN Mammas et al showed that there was no significant difference among children delivered through vaginal delivery or C-section. The C-section may not render protection against HPV infection as its transmission could occur transplacentally via amniotic fluid during gestation(20).

5. Conclusion

There was a significant detectable high risk HPV tonsillar infection of 8% prevalence in pediatric population. The association of maternal HPV risk factors including age, education level, mode of delivery and number of sexual partners was not statistically significant for the prevalence of HPV DNA. Large and more powered prospective studies are needed to study the association of risk factors and HPV positive tonsils. But also the ministry of Health in collaboration with other health sector stakeholders should provide education to the community on prevention of HPV infection.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declare that there is no competing interests in this work. No funding was provided by any financial or academic institution for this study. No conflict of interest to be disclosed.

Statement of ethical approval

The ethical clearance and approval for the study was sought from the Institutional Research Board from Muhimbili University of Health and Allied Sciences (MUHAS-REC-03-2021-516).

Statement of informed consent

Written informed consents were obtained from mother for sample collection from their children and data collection after understanding the all the information regarding the study. For those children above 7 years signed the assent form in addition to the signed of the consent form by their parents. Confidentiality was guaranteed. Administrative permission to conduct the study was obtained from Muhimbili National Hospital as per the hospital management protocols.

References

[1] Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Joneberg J, Creson N, et al. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. 2006;2623(September):2620–3.

[2] Garnaes E, Kiss K, Andersen L, Therkildsen MH, Franzmann MB, Filtenborg-barnkob B, et al. International Journal of Cancer to date. 2014;00(July):1–8.

[3] Anders N, Attner P, Hammarstedt L, Du J, Eriksson M, Giraud G, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm , Sweden : An epidemic of viral-induced carcinoma ? 2009;366:362–6.

[4] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Paula Curado M, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. J Clin Oncol. 2013;31(36):4550–9.

[5] Wojtera M, Paradis J, Husein M, Nichols AC, Barrett JW, Salvadori MI, et al. The prevalence of human papillomavirus in pediatric tonsils : a systematic review of the literature. 2018;1–8.

[6] Mujtaba H, Wang Y, Duan Y, Cao M, Zhang N, Murtaza A, et al. Pathology - Research and Practice Human papillomavirus in tonsillectomy specimen from China and Pakistan — Prevalence and genotype distribution. 2018;214(July):1713–8.

[7] Miller DL, Puricelli MD, Stack MS. Virology and molecular pathogenesis of HPV (human papillomavirus)-associated oropharyngeal squamous cell carcinoma. Biochem J. 2012;443(2):339–53.

[8] Ilmarinen T, Munne P, Hagström J, Haglund C, Auvinen E, Virtanen EI, et al. Prevalence of high-risk human papillomavirus infection and cancer gene mutations in nonmalignant tonsils. Oral Oncol [Internet]. 2017;73(April):77–82. Available from: http://dx.doi.org/10.1016/j.oraloncology.2017.08.010

[9] Hahn HS, Kee MK, Kim HJ, Kim MY, Kang YS, Park JS, et al. Distribution of maternal and infant human papillomavirus: Risk factors associated with vertical transmission. Eur J Obstet Gynecol Reprod Biol [Internet]. 2013;169(2):202–6. Available from: http://dx.doi.org/10.1016/j.ejogrb.2013.02.024

[10] Cason J, Mant CA. High-risk mucosal human papillomavirus infections during infancy & childhood. J Clin Virol. 2005;32(SUPPL.):52–8.

[11] Cockerill CC, Orvidas LJ, Moore EJ, Binnicker MJ, Duresko BJ, Espy MJ, et al. Detection of high-risk human papillomavirus infection in tonsillar specimens using 2 commercially available assays. Diagn Microbiol Infect Dis [Internet]. 2016;86(4):365–8. Available from: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.014

[12] Duray A, Descamps G, Bettonville M, Sirtaine N, Ernoux-Neufcoeur P, Guenin S, et al. High prevalence of high-risk human papillomavirus in palatine tonsils from healthy children and adults. Otolaryngol - Head Neck Surg. 2011;145(2):230–5.

[13] Mammas IN, Sourvinos G, Michael C, Spandidos DA. Human papilloma virus in hyperplastic tonsillar and adenoid tissues in children. Pediatr Infect Dis J. 2006;25(12):1158–62.

[14] Chen R, Sehr P, Waterboer T, Leivo I, Pawlita M, Vaheri A, et al. Presence of DNA of Human Papillomavirus 16 but No Other Types in Tumor-Free Tonsillar Tissue. 2005;43(3):1408–10.

[15] Palmer E, Newcombe RG, Green AC, Kelly C, Gill ON, Hall G, et al. Human papillomavirus infection is rare in nonmalignant tonsil tissue in the UK: Implications for tonsil cancer precursor lesions. Int J Cancer. 2014;135(10):2437–43.

[16] Ribeiro KMX, Alvez JM, Pignatari SSN, Weckx LLM. Detection of human papilloma virus in the tonsils of children undergoing tonsillectomy. Brazilian J Infect Dis. 2006;

[17] Smith EM, Parker MA, Rubenstein LM, Haugen TH, Hamsikova E, Turek LP. Evidence for vertical transmission of HPV from mothers to infants. Infect Dis Obstet Gynecol. 2010;2010.

[18] Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiavo KP. Perinatal transmission of human papilomavirus DNA. Virol J. 2009;6.

[19] Chatzistamatiou K, Sotiriadis A, Agorastos T. Effect of mode of delivery on vertical human papillomavirus transmission - A meta-analysis. J Obstet Gynaecol (Lahore). 2016;36(1):10–4.

[20] Mammas IN, Sourvinos G, Giamarelou P, Michael C, Spandidos DA. Human papillomavirus in the oral cavity of children and mode of delivery: A retrospective study. Int J STD AIDS. 2012;23(3):185–8.