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High Risk HPV Detected in Oral Cavity of Children in a Set Population of Karachi

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FI, SB and SS designed the study. Author FI collected the samples, did the bench work, wrote the protocol, and wrote the first draft of the manuscript. Author SB facilitated in bench work, Literature search and finalization of manuscript. Author MHL assisted in bench work and analyses of the data. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was designed to determine the frequency of HPV in school going children and also find out cytopathological changes in the oral mucosa resulting from HPV infection.
Study Design: Cross sectional study.
Place and Duration of Study: Samples of oral rinse were collected from 300 healthy school going children (aged 5-18 years) during the period of March 2014 to June 2014 from two school campuses of Karachi (South) Pakistan.
Methodology: Samples were divided into six ethnic groups according to mother tongue, including: Balochi, Pashto, Punjabi, Sindhi, Siraiki, and Urdu speaking. HPV was investigated using general primers (GP/5+ GP/6+) and HPV genotype kit (Genei eight high risk strains detection kit). For exfoliated cytology study slides were prepared and H&E staining was done to find out

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histopathological changes associated with HPV.
Results: Twenty three out of 300 (n=23/300) samples were positive representing 7.70% of the total screened. The 23 HPV positive samples included Balochi 4.3%, Pashto 13.0%, Punjabi 52.2%, Sindhi 0.0%, Siraiki 8.7%, and Urdu speaking 21.73% subjects. Further screening of high risk oncogenic genotype with Genei HPV kit yielded 6 (2%) positive samples, who were all females. None of slides was positive for any cytopathological changes.
Conclusion: The frequency of HPV was found 7.7% in school going children. The association between HPV infection, histological variables and tobacco use was not found.

Keywords: Human papilloma virus DNA tests; child; adolescence.

1. INTRODUCTION

Recently, a distinct section of youngsters with HPV positive status have been discovered in studies worldwide, and this is the population group which is less exposed to previously known risk factors like alcohol and tobacco [1]. While HPV is now being recognized as major independent factor among teenagers with a prevalence of 6.9%, in the United States threatening male gender more than females [2]. The incidence of oral cancer in Karachi is also on the rise. This is accredited to the local custom of tobacco chewing habit that leads to poor oral hygiene but also corrodes oral mucosa, which makes the mucosal surface conducive for HPV to gain entry into the basal layer of squamous epithelium [3].

HPV, a circular double-stranded DNA of approximately 8000 base pairs, is one of the most powerful human carcinogens present in the environment [3]. HPV can be classified into low and high risk types based on their capacity to promote malignant transformation in host cells. High risk types include HPV 16, 18, 31, 33, 35, 45, 52b, and 58; whereas, HPV 6, 11, 40, 42, 43, 89 etc. are considered as low risk viruses [4].

Previous studies on HPV targeted squatter settlements where mainly child labor is prevalent and schooling is not practiced. The aim of this study was to determine the frequency of HPV in school going children (in two squatter settlements) aged 5-18, and also to find out cytopathological changes in the oral mucosa resulting from HPV infection.

2. METHODOLOGY

This cross sectional study was conducted from March 2014 to June 2014 at Ziauddin University Karachi and it was a self-funded project. The study included all healthy children aged 5 to 18 years, selected from school settings. Excluded were that children who had any oral infection at the time of sample collection or if parents were not willing or School administration was reluctant to give informed consent.

Sample size was calculated using Epi Info version 6 software. The expected frequency was taken as 2.5% with confidence interval 95% and power 90. Oral samples were collected from 300 subjects after receiving informed consent. Ethical approval was obtained from The Ziauddin Ethics Review Committee. All experiments were performed in the postgraduate laboratory of Ziauddin University.

Sample collection was done through convenience sampling technique. Those schools were chosen who gave the consent for participation in the research study. From each grade 10 students were selected randomly through there roll numbers. The consent forms were given to the children one day prior to sampling for Parents consent and signature. All samples were collected as 20 - 40 ml oral rinse with gentle brushing over the oral mucosa with the help of a brush at the other end of dental floss and were stored at 4ºC until DNA extraction [5].

DNA was extracted and PCR was performed for HPV using general primers $Gp5^{+}/Gp6^{+}$ as previously described [5].

2.1 PCR for Oncogenic HPV Genotyping

PCR for oncogenic HPV detection was performed by using certified Genei™ HPV high risk typing kit (catalog # 610670100021730) according to manufacturer's protocol [6].

Data was entered on Statistical Package for Social Sciences (SPSS) version 20.0. Frequencies and percentages were taken out for the qualitative data; mean and standard deviation were taken out for the numerical variables.

Associations between qualitative variables were calculated using Pearson chi-square. At 95% confidence level, p-value less than 0.05 was taken as significant.

2.2 Sample Collection for Cytology

One end of a glass slide was labeled with subject's identity. A clean wooden spatula was used for collection of specimen. The spatula was moistened before scraping the oral mucosa. The specimen from oral mucosa was collected through slight rolling and scraping motions and smears were immediately spread on central area of the slide. After that Alcohol (70%) spray was used for fixation of the each smeared slide [5]. The smear was stained by a modified Papanicolaou-Traut technique using Mayer's Hematoxyline and Eosin [7].

3. RESULTS

Out of 300 enrolled subjects (aged 5-18yrs.)130 were females and 170 males (average age 13.60±10). They were divided into six ethnic groups according to mother tongue, including: Balochi [8(2.7%)], Pashto [47(15.7%)], Punjabi [127(42.3%)], Sindhi [15(5.0%)], Siraiki [48(16%)], and Urdu speaking [55(18.30%)] (Table 1).

HPV was positive in 4.3% of Balochi, 13.0% of Pashto, 52.2% of Punjabi, 0.0% of Sindhi, 8.7% of Siraiki, and 21.73% of Urdu speaking ethnicity. HPV was positive in 23 subjects (7.70%) out of 300. One hundred and eighteen Children (39.33%) were found using tobacco in any form.

Data also shows that habit of Brushing was not much prevalent in this age group (23%), and HPV was positive in 78.3% of those subjects who did not have regular brushing habits.

The screening with Genei HPV kit for oncogenic genotype of HPV revealed 6 out of 23 positive samples representing 2% of the total screened. A descriptive analysis of the Ethnic information regarding these samples revealed that all positive samples were from children belonging to Punjabi ethnicity (n=6/127 or 4.72%). Overall majority of HPV positive samples were from adolescents and teenagers and the 6 high risk genotype positive samples were all females.

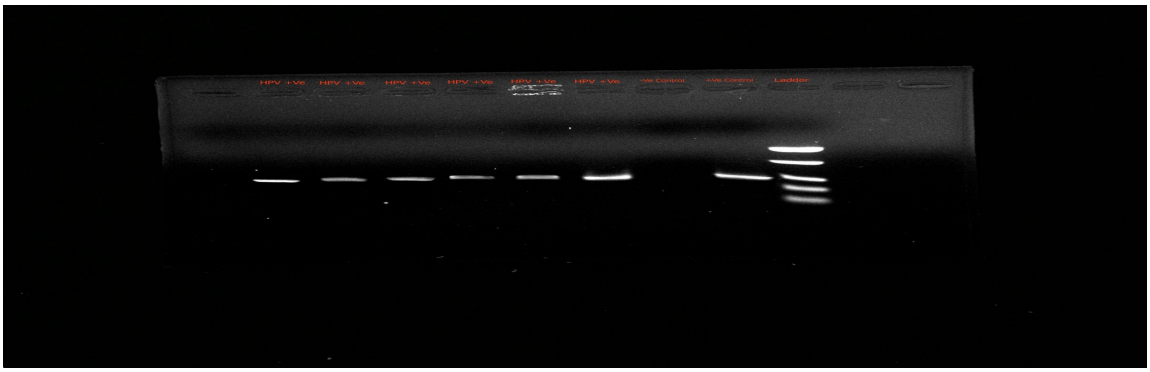
Screening for the cytopathological changes through exfoliative cytology slides & microscope examination showed no positive cytopathological changes.

4. DISCUSSION

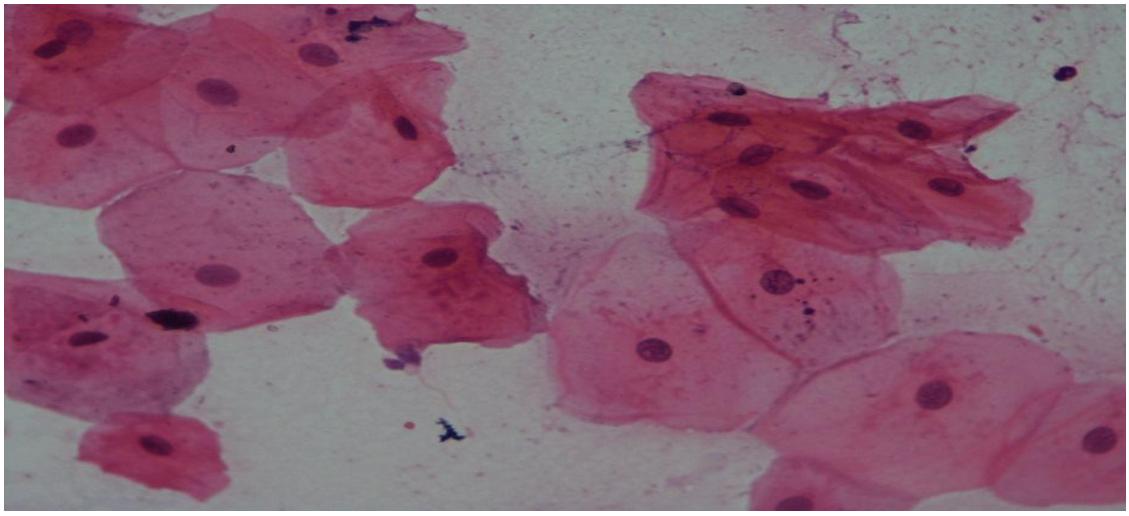
This is the first study from Pakistan reporting the frequency of HPV in school going children and adolescents. Children are more at risk of HPV associated infections, because of exposure to multiple sources of HPV vertical & horizontal transmission [8].In this study twenty three out of 300 (n=23/300) samples were positive for HPV ($Gp5^+/Gp6^+$), representing 7.70% of the total screened. This is proportional to a study done in Greece where 8.4% of 190 children were HPV positive and USA where 6% of the 268 participants were HPV-positive [9,10]. Majority (20 out of 23) of HPV positive (87%) subjects belonged to adolescent & teenagers group between ages 12 to 18 years, This is contradictory to a previous study by Summersgill et al. [10] who compared oral HPV prevalence between children and adults. They reported See comment in PubMed Commons belowHPV frequency as 8.7% in less than 7 years old whereas, 5.2% in 13 to 20 years old.

Table 1. Frequency of HPV in oral cavity of school going children seen in different ethnic groups

Variables	Punjabi n (%)	Urdu n (%)	Pashto n (%)	Siraiki n (%)	Sindhi n (%)	Balochi n (%)	Total n (%)
	127(42.3)	**55(18.30)**	**47(15.70)**	**48(16)**	**15(5.0)**	**8(2.70)**	**300(100)**
Male	69(54.33)	29(52.72)	31(56.36)	29(60.41)	6(40)	6(75)	170(56.7)
Female	58(45.66)	21(38.18)	16(34.04)	24(50)	9(60)	2(25)	130(43.3)
Gp5Gp6 +ve n=23	12(4)	5(1.66)	3(1)	2(0.67)	0(0)	1(0.33)	23(7.66)
Oncogenic HPV +ve n=6	6(100)	0(0)	0(0)	0(0)	0(0)	0(0)	6(100)
Cytopath. Changes	None	None	None	None	None	None	None



Agarose Gel Electrophoresis of human Papilloma virus, Polymerase chain reaction of six samples, stained with ethidium bromide and photographed under UV light (Fig. 1). Lane 2, 3,4,5,6 and 7 showing positive samples, Lane 8 denotes negative control while lane 9 displaying positive control. Lane 10 denoting molecular weight marker (Ladder)



H&E-stained cytological smear of healthy oral mucosa (Fig. 2) demonstrating normal squamous cells

Further screening for high risk oncogenic HPV genotype in this study revealed 6 (2%) positive samples. The genotype kit included eight high risk types (HPV-16, -18, -31, -33, -35, -45, -52b, and 58) which have been considered as high risk by World Health Organization (WHO) International Agency for Research on Cancer (IARC) [11]. All 6 high risk positives were teenager females and this age has also been found to be at high risk by other studies [9]. This association of HPV with female gender (60%), though the values were not significant (P value of <0.077) requires further investigations. This is in contrast to previous studies in Pakistan where prevalence of HPV was higher in males than females [12].

Three (13%) low risk positive children belonged to age group between 2 to 11 years. Although, HPV in this regard was not further genotyped, however, other studies in this age group have found HPV 6 and 11 as the most common genotypes [9,13]. It is generally agreed that HPV is prevalent in oral samples of newborns of the infected mothers [14]. In children, mainly, less than four years of age, close maternal-newborn concordance result as major transmitter of HPV from the mother to their newborn. HPV infections are acquired horizontally via saliva or through HPV DNA transmitted post-nattily during bathing or diapering [13].

Tobacco use (in the form of smoking as well as chewing) in school going children could be one of the leading reasons of HPV association in adolescents, but this study did not reveal any significant relationship with chewable tobacco in these subjects who were using it in any form even for a longer duration, though longer exposure of tobacco can lead to increased expression of oncogenic HPV [15].

A probable explanation of finding HPV in school going children could possibly be due to poor oral hygiene, which produces viable environment for HPV to gain entry into the basal layer of oral mucosa. The information regarding alcohol drinking and oral sex was not determined from this study population since it is prohibited according to the Islamic laws.Further larger studies are required with larger sample size to determine factors that affect HPV status.

The persistence of HPV in oral cavity is not yet documented. Rintala et al suggest that the reservoir for HPV in the oral mucosa may be gingival pockets in emerging teeth [16].

The analysis of the morphological changes in the cells is a gold standard for cytopathology and has vast implications in oral pathology [7]. Examination of oral mucosa for cytopathological changes in healthy school going children could provide vital information regarding the pathological changes due to HPV. The present study also offered use of main diagnostic application of exfoliated cytology in relation to HPV infection but none of the slides were positive for any cytopathological changes. This suggests that cytopathological changes develop with long term persistent infection which might be the reason for not finding any abnormal morphology in these subjects.

5. CONCLUSION

The frequency of HPV was found 7.7% in school going children, out of which 6 (2%) of children were positive for HPV high risk strains. The association between HPV infection and histological variables and tobacco use was not found**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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