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Prevalence of oral HPV infection and genotype distribution in Iranian children during the early ages of life

Mona Sadat Larijani[1], Amir Javadi[2], Alireza Fahimzad[3], Rahim Soleimani[4], Farbod Tabatabaei[5], Amir Houshang Nejadeh[6], Mahboubeh Jamshidi[6], Anahita Bavand[1], Ladan Moradi[1], Fatemeh Ashrafian[1*] and Amitis Ramezani[1*]

Abstract

Background Human Papillomavirus (HPV) infection in the oral cavity has been shown to be common in the young populations with uncertain consequences. Persistent oral oncogenic HPV infection could lead to oropharyngeal malignancy. HPV transmission from mother to fetus is still under question besides the horizontal routes which seem to be emerging. Although HPV infection is mostly transient in young populations, detecting oral HPV in children might be valuable to understand the prevalence, transmission, and natural history of HPV infections in this age group and determination the role of maternal vaccination against HPV. The present study aimed to determine the oral HPV prevalence and genotyping in Iranian children during first-5-year of life for the first time.

Methods This cross-sectional study was conducted in Pasteur Institute of Iran. Buccal samples of the population ≤ 5 years old and also of a subset of mothers were investigated. DNA extraction was done and Real-Time PCR was performed to characterize HPV infection. The positive samples were re-assessed through hybridization assay.

Results Totally, 201 children aged from 3 days to 5 years old were enrolled in this study among whom16 children were HPV-positive accounting for 7.9% (n=16) with a higher incidence in the population <1month (27.3%). HPV16 and HPV18 were the most frequent HPV types accounting for 50% and 37.5%, respectively followed by HPV31, HPV35, HPV39 and HPV56. Multiple HPV infections were detected in 7 children among whom HPV16 was dominantly detected (71.4%). In a subgroup of mothers who provided oral samples, the total HPV prevalence was 9.5% and HPV18 was the most frequent type followed by HPV16, HPV82, HPV35 and HPV11. Furthermore, the positive HPV status in mothers led to a significant risk of infection in children (p<0.001; OR=165). HPV genotypes between mothers and offspring did not show full concordance. What is more, a significant difference regarding the type of delivery and HPV positivity in children was observed (p<0.001).

Conclusion The present research indicates that oral HPV infection is quite common in early ages of Iranian children. Multiple HPV infections and a high prevalence of HPV16 are concerning issues for unknown consequences. Lack of full concordance of HPV types between mothers and offspring highlights the possible routes of horizontal transmission. Detecting oral HPV, especially the oncogenic types, could provide rationales for screening tests and setting

*Correspondence:
Fatemeh Ashrafian
Fatemeh.ashrafian24@gmail.com
Amitis Ramezani
amitisramezani@hotmail.com
Full list of author information is available at the end of the article

policies in the future. Additionally, the obtained results emphasize HPV vaccination programs to reduce the rate of HPV transmission from mothers to children.

Keywords HPV infection, Oral cavity, Oropharyngeal carcinoma, Buccal sample, HPV concordance, Children

Introduction

Human Papillomavirus (HPV) belongs to the Papillomaviridae family with over than than 200 different types of the virus existing. HPVs are categorized according to their epithelial tropism, including cutaneotropic and mucosotropic viruses [1] as well as their phylogenetic relationship [2, 3]. Therefore, these viruses could manipulate different parts, including the skin, anogenital tract and oral cavity [4, 5]. HPV, is primarily known as the main cause of cervical cancers and subsequently thousands of associated deaths worldwide. Nevertheless, it also plays a crucial role in oral lesions such as verrucapapillary lesions, multifocal epithelial hyperplasia and oral squamous cell carcinoma [6–8]. Cutaneous warts which are mostly acquired through horizontal transmission, are common in children and can persist asymptomatically for years [9]. In contrast to HPV cutaneous manifestation, mucosal infections generally stem from sexual transmission. Notably, definite mucosal HPV types, belonging to α-HPVs, have been detected in nonsexually exposed populations, including infants and children, in oral mucosa, indicating a nonsexual route of transmission [10, 11]. This type of transmission could be through vertical or horizontal modes [12]. Notably, current evidence demonstrates the presence of high-risk HPV infection in normal children with the highest prevalence in the population younger than 7 years old [13, 14]. According to published data, it could be assumed that oral HPV acquisition occurs through close contact with parents, family members or fomites as well as contact with infected vectors in preschool or daycare centers [15, 16]. However, the primary exposure to HPV infection in children and infants is considered to occur through passing the infection in placenta or vaginal canal during delivery [17].

In a recent meta-analysis study [18], of the 2638 children aged from 1 day to 12 years old investigated between 1994 and 2021, the total prevalence of HPV infection is 14.7%. HPV infection could manifest in oral cavity through different ways including oropharynx squamous cell papilloma which is benign and mostly occur in middle ages of life though it may also present in children ≤10 years old accounting for 7.9% of oral tumors in this population [19, 20]. Furthermore, recurrent respiratory papillomatosis is also a presentation by this virus, mainly HPV types 6 and 11, among children aged 2 to 6 years old [21].

HPV manifestations in the oral cavity have been investigated in children through different approaches though the possible consequences of the infections have not been known yet. There are no established guidelines for screening oral HPV infection. Nevertheless, detecting oral HPV in children might be valuable to understand the prevalence, transmission, and natural history of HPV infections in this age group. Identifying HPV in children could provide us with routes of transmission and consequently inform preventive measures. Furthermore, determination HPV infections is rational to achieve cumulative data to set further guidelines and policies, especially in the populations in which vaccination against HPV is not well-established and the level of awareness requires fundamental modifications.

In this study for the first time, we aimed to assess the oral HPV prevalence and genotyping in Iranian children during the first 5 years of life.

Matherial and methods

This cross sectional study was approved by Pasteur Institute of Iran National Committee for Ethics (Ethical No. IR.PII.REC.1402.061) and performed in collaboration with pediatrics hospitals of Teharn from March to October, 2024. The population ≤ 5 years old who saought medical care, routin checkup or vaccination were included in the study upon an interview with their parents and signing the consent form. The children who had acute condition were not included.

The demographic data were collected from parents by using designed questionnaires including the type of delivery, breast feeding status, medical history, number of children and the order of birth.

Furthermore, during the interview with parents, the mothers who agreed to participate, provided baccual samples along with the offsprings.

Buccal samples were performed using sterile nasopharyngeal flocked swabs (Hakimane Ofogh Pars Sabzine, Iran). Each collected swab was then placed in viral transport medium and stored at −20°C. DNA extraction was done using SENMURV Extraction Nucleic Acid kit (BONEX-100, STEMCELL THECKNOLGY, Iran). The purity and concentration of the extracted DNA was then assessed using Nano-drop (Denovix, USA). Real-Time PCR was applied to characterize HPV infection using SENMURV HPV Genotyping

kit [BONHPV2-100, STEMCELL THECKNOLGY, Iran [22]. This HPV kit is designed in two tubes in order to detect 14 common types of high-risk human papilloma virus (including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and two common types of low-risk HPV, types 6 and 11, in swab samples. Beta globin primers, included in the kit as internal control [23]. According to this test, HPV detection could be achieved by a two-reaction test and so, some HPV types are detected in one reaction. Therefore, to confirm and specify HPV genotypes solely, positive samples were re-assessed through CLART® HPV4 hybridization assay (GENOMICA, Spain) which detects HPV genotypes 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68a and b, 70, 71, 72, 73, 81, 82, 83, 84, 85 and 89. PGMY09/PGMY 11 primers for HPV L1 gene and CFTR primers as internal control were provided by the kit [24].

Statistical analysis

Shapiro-Wilk test was used to evaluate the normality of numerical variables. The numerical data are summarized as mean ± SD (standard deviation) and categorical data as frequency (Percentage). For numerical variables, comparison between two groups was done using Independent T-test.

Chi-square test (Fisher's exact test) was used to investigate the relation between categorical variables. Variables which had a *p*-value of equal to or less than 0.20 in the bivariate analysis were included in the multivariate analysis. The adjusted odds ratio (OR) with 95% confidence interval (CI) was calculated by multiple logistic regression (MLR) analysis. The Wald test was used to examine the effect of each independent variable on the HPV status. A Chi-square test was used to determine significant differences between proportions. SPSS version 18.0 (Chicago, IL, USA) was used for all statistical analyses. The level of significance was less than 5% for all statistical tests.

Results

Study population

Totally, 201 children including 106 girls and 96 boys, aged from 3 days to 5 years old were enrolled in this study.

The study population was classified according to the age range as following:

The population younger than 1 month (*n*=11), the population aged 1 month to 6 months (*n*=38), the population aged 6 months to 12 months (*n*=42), the population aged 1 year to 3 years old (*n*=60), the population aged 3 years to 5 years old (*n*=50).

The order of birth showed that 46.3% of the population study were the first or only child of the family, followed by 34.3%, 13.4% and 6% as the third, second and fourth children, respectively (Table 1). The vast majority of the children were born through cesarean section whereas 21.4% by vaginal delivery. Furthermore, 85.1% (*n*=171) of the individuals were breastfed. The medical history also included seizure (*n*=5), lactose intolerance (*n*=3), gastroesophageal reflux (*n*=3), Clubfoot (*n*=2), hypothyroidism (*n*=1), Minor Thalassemia (*n*=2), Favism (n=1), Cardiac arrhythmia (*n*=1), LCH: Langerhans Cell Histiocytosis (*n*=1), meningitis (*n*=1), Laryngomalacia (*n*=1), PFAPA (Periodic fever, aphthous stomatitis, pharyngitis and adenitis) Syndrome (*n*=1), Asthma (*n*=1) and Transposition of the Great Arteries (*n*=1). What is more, 4 kids had a previous history of NICU (neonatal intensive care unit) care. The familial history also indicated that 31.3% (*n*=63) of the studied children had a positive background of a malignancy among immediate or extended family. Nevertheless, none of the HPV-infected children had a familial history of cancer.

HPV detection and genotyping

Totally 16 children were detected HPV-positive via Real-Time PCR and CLART HPV4 hybridization technique (Table 1, Fig. 1). The total HPV prevalence among the studied population accounted for 7.9%.

There was no significant difference regarding HPV status between boys and girls (*P*=0.447). According to the positive cases in each age group, the infants younger than 6 months showed a high prevalence of HPV accounting for 27.3% (*n*=3). On the contrary, children aged from 3 to 5 had a lower rate of HPV prevalence (0.049). Furthermore, there was a significant difference regarding the type of delivery and HPV status in children ($p<0.001$). We did not observe any significant association between the HPV status and being breast-fed (*P*=0.86).

As shown in Table 2, HPV16 and HPV18 were the most frequent HPV types accounting for 50% (*n*=8) and 37.5% (*n*=6), respectively followed by HPV31 (*n*=2), HPV35 (*n*=2), HPV39 (*n*=1) and HPV56 (*n*=1). Low-risk HPV6 was detected in 4 cases (25%) whereas HPV11 in only one child (6.3%).

Multiple HPV infections were seen in 7 children and notably, HPV16 was detected in 71.4% (*n*=5) of them, mostly accompanied by HPV18 (*n*=3, 60%).

Maternal HPV status and its comparability to offspring

In a sub-population of the children, oral HPV infection was assessed in the mothers in addition to their children (Table 3, Fig. 2). Totally, 74 mothers with a mean age of 34.16±5.28 (min: 23, max: 45) provided samples. Three individuals had a kind of underlying diseases including Diabetes Mellitus, hypothyroidism and arthrosis. 94.5% (*n*=70) of this population had an academic degree though only a minority (*n*=7) of them were familiar with HPV

Table 1 The characteristics of the studied population and the impact of different variables on HPV infection

Variable	Total	Negative	Positive	OR (CI%)	*p*-Value*
n (%)	201	185 (92.0%)	16 (7.9%)		
Sex					
Female (Ref.)	106 (52.7%)	96 (90.6%)	10 (9.4%)	-	
Male	95 (47.3%)	89 (93.7%)	6 (6.3%)	1.55 (0.54–4.43)	0.447
Age					
< 1 Month (Ref.)	11 (5.5%)	8 (72.7%)	3 (27.3%)	-	
1–6 Months	38 (18.9%)	38 (100%)	-	-	-
6–12 Months	42 (20.9%)	38 (90.5%)	4 (9.5%)	0.28 (0.05–1.51)	0.138
1–3 Years	60 (29.9%)	54 (90.0%)	6 (10.0%)	0.30 (0.06–1.43)	0.129
3–5 Years	50 (24.9%)	47 (94.0%)	3 (6.0%)	0.17 (0.03 –1)	**0.049**
Birth order					
1 (Ref.)	93 (46.3%)	86 (92.5%)	7 (7.5%)	-	
2	27 (13.4%)	25 (92.6%)	2 (7.4%)	0.98 (0.19–5.03)	0.974
3	69 (34.3%)	63 (91.3%)	6 (8.7%)	1.17 (0.38–3.65)	0.787
4	12 (6.0%)	11 (91.7%)	1 (8.3%)	1.12 (0.13–9.95)	0.921
Delivery type					
Vaginal (Ref.)	43 (21.4%)	34 (79.1%)	9 (20.9%)		
Cesarean section	158 (78.6%)	151 (95.6%)	7 (4.4%)	0.18 (0.06–0.50)	**<0.001**
Breast feeding					
No (Ref.)	30 (14.9%)	28 (93.3%)	2 (6.7%)		
Yes	171 (85.1%)	161 (94.2%)	14 (8.1%)	0.86 (0.19–4.18)	0.86
History of underlying diseases					
No (Ref.)	171 (85.1%)	156 (91.2%)	15 (8.8%)		0.475
Yes	30 (14.9%)	29 (96.7%)	1 (3.3%)	0.36 (0.05–2.82)	

* Chi-square and Fisher's exact tests were used to investigate the relation between categorical variables. Significant values are presented in bold

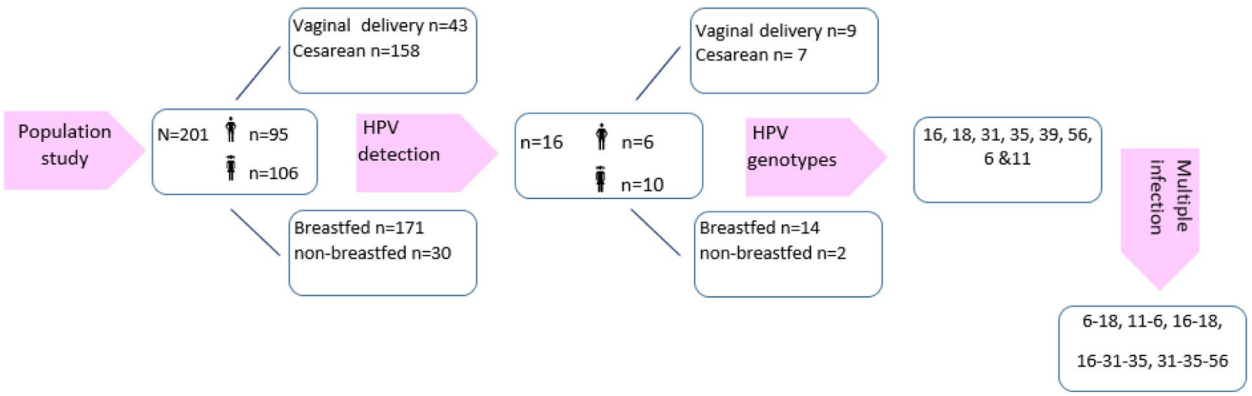

Fig. 1 Children study population's characteristics and the associated HPV status. Totally 201 children were assessed to detect oral HPV infection. As presented in figure, 16 kids were HPV positive. The detected HPV genotypes showed that multiple infections were also present in this population, mostly with HPV16

infection or adhered to a routine HPV screening schedule. According to the obtained data, only 3 subjects (4%) had received Gardasil vaccine and none of the subjects declared to have any gynecological problems up to the time of the study.

Table 4 presents the total HPV prevalence among the mothers which accounted for 9.5% (n=7). The HPV

Table 2 HPV prevalence and genotypes in the studied children

Variable	Positive	Type (number of cases)
n (%)	16 (8.0%)	
Number of HPV genotype		
One	9 (56.3%)	HPV6 (3), HPV16 (3), HPV18 (2), HPV39 (1)
Two	5 (31.3%)	HPV6-18 (1), HPV11-16 (1), HPV16-18 (3),
Three	2 (12.5%)	HPV16-31–35 (1), HPV31-35–56 (1)
Low risk HPV		
HPV 6	4 (25.0%)	
HPV 11	1 (6.3%)	
High risk HPV		
HPV 16	8 (50%)	
HPV 18	6 (37.5%)	
HPV 31	2 (12.5%)	
HPV 35	2 (12.5%)	
HPV 39	1 (6.3%)	
HPV 56	1 (6.3%)	

Table 3 Characteristics of the studied mothers and their children

Variable	Total	HPV Status		OR (CI%)	*p*-Value*
Subgroup of mothers and children	**Number (mothers)**	**Negative**	**Positive**		
n (%)	74 (100)	67 (90.5%)	7 (9.5%)		
HPV Vaccination					
No	71 (95.9%)	64 (95.5%)	7 (100%)		0.999
Yes	3 (4.1%)	3 (4.5%)	-	-	
Children's gender					
Female (Ref.)	43 (58.1%)	38 (88.4%)	5 (11.6%)		
Male	31 (41.9%)	29 (93.5%)	2 (6.5%)	0.52 (0.10–2.89)	0.229
Kids' HPV status					
No (Ref.)	68 (91.9%)	66 (97.1%)	2 (2.9%)		**<0.001**
Yes	6 (8.1%)	1 (16.7%)	5 (83.3%)	165 (12.7–2149)	

* Chi-square and Fisher's exact tests were used to investigate the association between categorical variables. Significant values are presented in bold

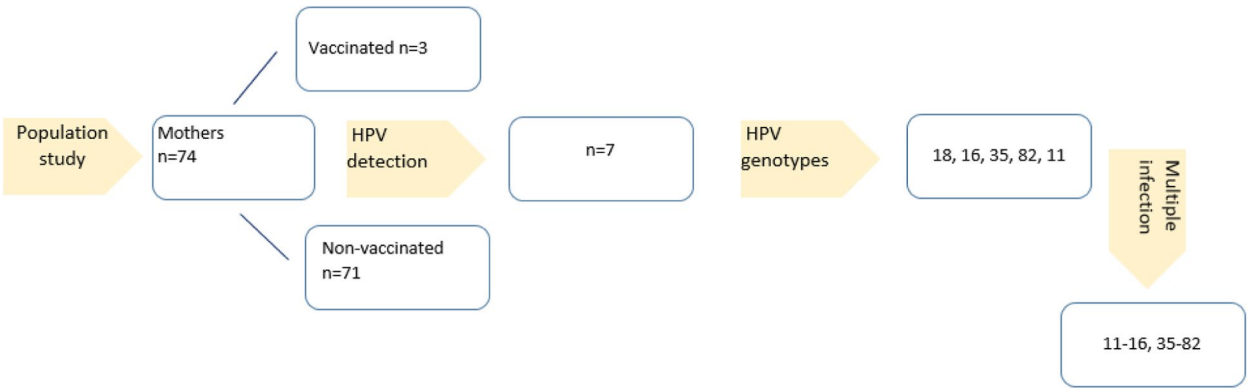

Fig. 2 Mothers population study and the associated HPV status. Totally 74 mothers provided oral HPV samples in addition to their children. As presented in the figure, 16 kids were HPV positive. The detected HPV genotypes showed that multiple infections were also present in this population, mostly with HPV16

Table 4 Evaluation of oral HPV prevalence in the studied mothers

Variable	Positive	Type
n (%)	7 (9.5%)	
Number of HPV genotype		
One	5 (71.4%)	HPV16 (1), HPV18 (3), HPV82 (1)
Two	2 (28.6%)	HPV11-16 (1), HPV35-82 (1)
Low risk HPV		
HPV 11	1 (14.3%)	
High risk HPV		
HPV 16	2 (28.6%)	
HPV 18	3 (42.9%)	
HPV 35	1 (14.3%)	
HPV 82	2 (28.6%)	

genotyping showed that HPV18 was the most frequent type (n=3) followed by HPV16 (n=2), HPV82 (n=1), HPV35 (n=1) and HPV11 (n=1). It should be noted that one of the mothers was co-infected with HPV16 and HPV11.

There was a significant difference between kids' HPV status born to infected mothers compared to those born from healthy women. In fact, of 6 HPV-positive kids (born from 74 providing sample mothers), 83.3% (n=5) were from HPV-infected mothers. In other words, the positive HPV status in mothers led to a significant rate of infection risk in children (p<0.001; OR=165). Notably, there was a child with HPV16 born to a negative-HPV mother. The data also indicated that a mother with HPV16, had a daughter with multiple HPV infections including HPV16 at the age of 4 years.

The comparison between HPV genotypes between children and mothers with HPV, are presented in Fig. 3. Of the five pairs of mothers and offspring, HPV was detected exactly the same in two pairs for HPV18 in one pair, and HPV 16-11 in the other one. Notably, three kids had a different HPV type from maternal parent standing for HPV6, 56, 31 and 35 (Fig. 3). In two multiple infected children, only one type was compatible to their mothers. HPV 16 and HPV18 were the most common types between the mothers and children.

Discussion

HPV infections in oropharyngeal cavity may lead to unsolicited outcomes in young populations which is still under question. HPV is mostly considered as a vertically transmitted infection and therefore it could manifest in newborns during the delivery [12, 25]. In addition to this route, some studies have shown periconceptional HPV infection owing to the fact that HPV DNA was detectable in spermatozoa, seminal fluid, ovaries and endometrium [26, 27]. Although different studies have

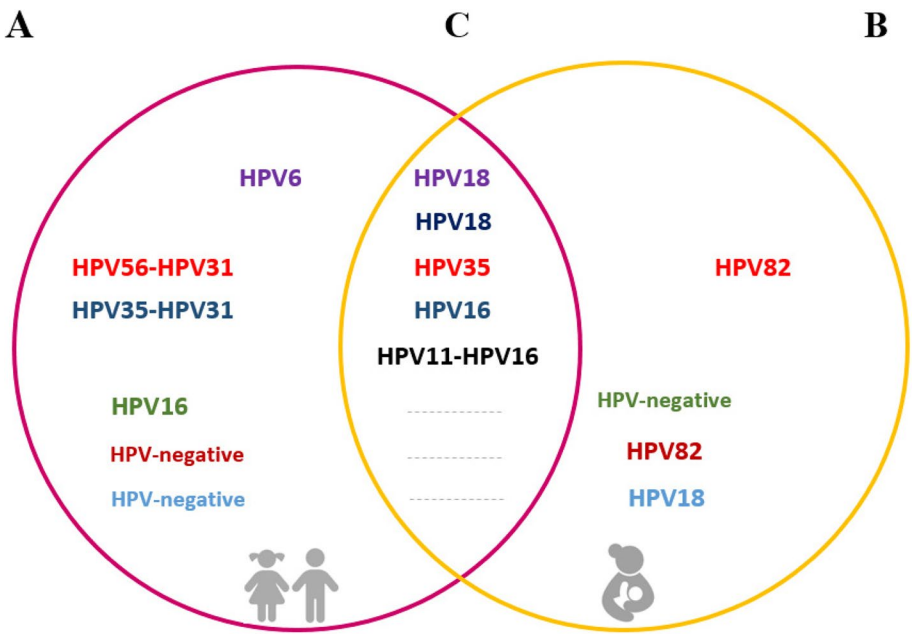

Fig. 3 Comparability of HPV genotypes of mothers to offspring. This figure presents the concordance of children with HPV, whose mothers' oral samples were investigated. Each color presents a pair of child and mother. **A** shows the HPV status in children (of the positive mothers). **B** Illustrates the seven mothers with oral HPV infection. **C** Presents the shared HPV status between mothers and offspring. The second and fourth pairs have exactly the same HPV types. Two mothers with HPV had healthy children and a kid with HPV18 was born to a healthy mother. The dotted lines in zone C indicates no concordance between the mother-child pairs

investigated HPV prevalence in young populations, the heterogeneity in terms of age, sample type, maternal/paternal inclusion and affecting variables; is remarkable. Therefore, it seems crucial to investigate HPV presence during early ages of life to achieve more accurate evidence in this regard.

In this study which is the first evaluation of oral HPV infection in Iranian children, the total HPV prevalence in oral samples was 7.9% and high-risk HPV types; 16 and 18 were the most frequent ones.

The analyzed data showed no significant association regarding children's HPV status and gender, birth order and underlying diseases. It has been suggested that HPV could be vertically transmitted by breastfeeding [28]. Although he vast majority of the children in the present investigation were breast-fed, there was no significant association between this variable and HPV infection in them. Louvanto et al., investigated breast milk and detected HPV DNA in it during different sampling time which persisted among 5.5% the lactating mothers. However, they did not find any significant associations between the breast milk HPV and the infants' oral HPV infection [29].

Interestingly, multiple HPV infections were detected in 43.75% (n=7) of positive-HPV children in whom HPVs16 and 18 were frequent and accompanied by other types. Furthermore, maternal oral HPV infection showed that 9.5% of the studied mothers were HPV-positive among whom 28.6% were infected with more than one type. The comparison between HPV genotypes in HPV-positive kids and mothers, showed that only 2 pairs (of 5) shared the same HPV types.

The estimated total HPV in this study is in line with Finnish study in which oral HPV for varied from 8.7% to 22.8% depending on the time of sampling [30]. In agreement with our findings, the association between mothers' oral HPV infection and positive HPV status in children has been confirmed in the Finnish study [30]. In addition, a meta-analysis including 3128 mothers and children stated that children born to HPV-positive mothers were 33% more likely to be HPV positive than children born to HPV-negative mothers [9]. In a study by Castellsagué et al. in Spain, there was an association between mothers and kids' HPV status. They found that the children born from HPV-positive mothers were 5 times more likely to HPV acquisition 6 weeks post-partum [31]. We also observed that 83.3% of HPV-infected children were born from HPV-positive mothers and the positive HPV status of mothers was significantly related to children's HPV positivity. In agreement with the present data,, SkoczyNski et al., declared that oral HPV infection has an 11-time higher relative risk in children of infected mothers with oral/cervical HPV [32].

Park et al., found an association between HPV transmission to kids and vaginal delivery [33]. We also found that there was a significant association between the vaginal delivery and HPV status in kids ($P = 0.005$). Furthermore, in recent research, Nantel et al., declared that vaginal delivery resulted in an increased risk of HPV transmission to infants compared to caesarean section [34].

Totally in HPV positive cases 8 different HPV genotypes were detected in the present data including 16, 18, 31, 35, 39, 56, 6 and 11. In the Finnish study, 18 different HPV types were identified in the oral samples. In line with our findings, HPV16 was the most common genotype, followed by HPV18 [30]. According to the follow-up schedule, they found multiple-HPV infections ranging from 0.3% to 3.7% during the six years post-birthday. In the present population study, 43.7% of HPV-infected kids had multiple infections and HPV16 was the most prevalent type.

An Indian study conducted by Bandyopadhyay S et al., showed that HPV16 had the highest prevalence in both HPV-positive maternal and offspring's samples [35]. Although HPV16 was the most common type in oral samples of studied children in the present data, in maternal samples HPV18 showed dominance followed by HPV16.

In a study conducted by Puranen et al., of the HPV-positive nasopharyngeal samples obtained from newborns, the concordance between HPV types in the genital tract of mothers and newborn was 69% [36]. In a study by Koskimaa et al., the HPV genotype between mothers and offspring was the same at delivery time, however, this concordance disappeared in 2 months [37]. Of the five pairs of mothers and offspring infected with oral HPV in the present study, HPV was detected exactly the same in only two pairs. One kid was <30 days and the other one was about four years old. On the other side, three kids had a different HPV type from maternal parent standing for HPV6, 56, 31 and 35 of whom one was younger than 30 days and the others were 2 years and three years old. These data together support the hypothesis that HPV could be transmitted through horizontal routes and lead to oral mucosa infection of the newborn. The lack of concordance between children's oral HPV type and the mothers, could stem from different modes of HPV transmission such as household contact, environmental exposure and close contact to other family members and caregivers [38, 39].

It should be considered that HPV infections are often transient and could be naturally cleared in mothers within months to a few years [40]. Therefore, maternal parents might have had HPV in the past, but not at the time of sampling. The other possibility is the different site

of sampling. In this study, we did not collect cervical samples in which the oral strain of an infected child might be present. Some HPV types prefer the oral mucosa, while others persist better in the genital area and so, some HPV types might have an affinity for certain epithelial tissues due to differences in cell receptors [41]. Thus, the low HPV concordance rate, does not rule out maternal transmission, but it highlights other sources or possible natural clearance of previous HPV infection in mothers.

HPV detection and genotyping of high-risk types in the oral cavity could be important since the persistent infection which mostly stems from HPV16 could increase the risk of further head and neck cancers. A systematic review by Kreimer et al., showed the presence of HPV DNA in 35.6% of oropharyngeal malignancies in which HPV16 accounted for 87% of all HPV-positive individuals [42].

Oral HPV monitoring has not been considered yet according to the lack of criteria and required necessity. This issue needs a sensitive and specific screening test for population who are identified risky ones including children from HPV-positive status. This screening could result in early diagnosis and also mortality reduction based on applying preventive interventions focusing on managing existing infections, preventing future infections, and promoting overall health. From another point of view, the vast majority of the mothers had academic education but only a minority of them had a routine HPV screening schedule. Furthermore, 4% of this population had received Gardasil vaccine. Therefore, studies which aim to assess HPV infection would contribute to the awareness regarding the routes of HPV transmission and also the importance of vaccination at the proper time. The present study and similar data could contribute to achieve the data whether the detection of oral HPV infections are necessary, especially in young populations, and also lead to set an appropriate screening program for the target population.

This study has the strength of oral HPV detection in children and a subset of their mothers in Iran for the first time. Nevertheless, it is a cross-sectional study and therefore the discussed possible dynamics of transmission is speculative. It lacks some information including all the related mothers, genital sampling and follow-up data. Furthermore, the research setting did not provide a follow-up schedule to estimate HPV persistency in children.

Conclusion

To sum up, the present data clearly shows that oral HPV infection is slightly common during the first-five years and the oral cavity is an important site of HPV exposure. Furthermore, multiple HPV infections and the high prevalence of HPV16 in the studied population raise concerns for further consequences. What is more, lack of full concordance HPV types between mothers and offspring is controversial and highlights the possible routes of horizontal transmission. The present data might contribute to highlight the importance of oral HPV screening especially in young populations from HPV-positive mothers. It also indicates the necessity of education towards HPV infection and prevention. Further studies including different age ranges with long-term follow-up schedule is recommended.

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Authors' contributions
Mona Sadat Larijani: Contributed to the study conceptualization and design, laboratory tests validation, original draft writing and revising; Amir Javadi: Contributed to the data analysis, Alireza Fahimzad: Contributed to cases visiting and providing samples; Rahim Soleimani: Contributed to Real-Time PCR optimization and analysis; Farbod Tabatabaei: Contributed to laboratory tests and data entry, Amir Houshang Nejadeh and Mahboubeh Jamshidi: Provided hybridization assays and data validation; Anahita Bavand and Ladan Moradi: Contributed to data collection and handling the cases, Fatemeh Ashrafian: Contributed to case management, data entry and project administration; Amitis Ramezani: Contributed to conceptualization and study design, revision of the manuscript and data validation.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All the individuals were provided by informed consent prior to enrolment and the protocol was performed according to the Declaration of Helsinki (Fortaleza, 13 October 2013).
The study protocol was approved by the Pasteur Institute of Iran National Committee for Ethics (Ethical No. IR.PII.REC.1402.061)

Competing interests
The authors declare no competing interests.

Author details
[1]Clinical Research Department, Pasteur Institute of Iran, Tehran, Iran. [2]Department of Community Medicine, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran. [3]Division of Pediatric Infectious Diseases, Department of Pediatrics, Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [5]Faculty of Converging Science and Technology, Islamic Azad University, Tehran, Iran. [6]Department of Pathobiology and Pathology, Ayandeh Pathobiology and Genetics Laboratory, Varamin, Iran.

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