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ORIGINAL ARTICLE

Prevalence of Human Papillomavirus (HPV) in upper respiratory tract mucosa in a group of pre-school children

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Abstract

Introduction. Human Papillomavirus (HPV) is a group of DNA viruses which is an etiological factor of many benign and malignant diseases of the upper respiratory tract mucosa, female genital tract and the skin. HPV infection is considered a sexually-transmitted infection, but can also be transmitted by non-sexual routes, including perinatal vertical transmission, physical contact, iatrogenic infection and autoinoculation. Recurrent Respiratory Papillomatosis (RRP) in children is connected with HPV infection transmitted vertically from mother to child during the passage of the foetus through an infected birth canal.

Objective. The aim of this study was to establish the level of Human Papillomaviruses carrier state in upper respiratory tract mucosa in healthy pre-school children, and to identify potential risk factors for HPV infection.

Materials and Method. After obtaining consent from their parents, 97 pre-school children were examined – 51 girls and 46 boys between the ages of 3 – 5 years; average age – 4 years and 5 months. 68 children were urban dwellers and 29 came from a rural environment. A questionnaire with detailed history was taken including parents' and child`s personal data, as well as perinatal risk factors in pregnancy. Socio-demographic information was also obtained, including the standard of living, and chosen environmental factors. Routine ENT examination was performed. Exfoliated oral squamous cells were collected from swabs and analysed for the presence of DNA papillomaviruses by polymerase chain reaction.

Results. The presence of HPV in the respiratory tract in children was detected in 19.6% cases. 'High oncogenic potential' HPVs, such as HPV-16 and HPV-18, were not observed in squamous cell mucosa of the respiratory tract in the children. No significant differences were observed between the HPV carrier state in urban and rural inhabitants.

Key words

HPV infection, respiratory tract, PCR.

INTRODUCTION

Human Papillomavirus (HPV) is a group of DNA viruses which is an etiological factor of many benign and malignant changes in the upper respiratory tract mucosa, female genital tract mucosa, and the skin [1, 2]. The virion of HPV is small – 52–55 nm in diameter. Capsid is built of 72 capsomeres (L1 and L2 conservative proteins), inside which there is a round, double-stranded DNA molecule ca. 8 kbp long. To date, more than 150 different HPV virus genotypes have been identified, differentiated by the genetic sequence of the outer capsid protein L1 [3, 4].

All HPV can be divided into 2 main groups: 'low risk' (HPV-6) or 'non-oncogenic' (HPV-11), and are connected with skin warts, papillomas of the mucosa of the oral and nasal cavity, pharynx, larynx, and genital tracts, which most frequently are changes of a non-malignant nature. The second group consists of those with high oncogenic potential HPV (HPV-16 and HPV-18), linked with neoplastic changes (identified in about 98% of cervical cancers). 'High risk' HPV infection also plays a role in the pathogenesis of squamous cell carcinomas of the head and neck, especially those arising in the oropharynx, tongue and tonsils. Approximately 4% of all cancers are associated with HPV [1, 5, 6, 7, 8, 9, 10, 11].

Although HPV infection is considered a sexually-transmitted infection, HPVs can also be transmitted by non-sexual routes, including perinatal vertical transmission, casual physical contact, iatrogenic infection and autoinoculation. Many years ago it was noticed that Recurrent Respiratory Papillomatosis (RRP) in children may be caused by the Human Papillomavirus transmitted vertically from mother to child during passage of the foetus through an infected birth canal. However, this finding has not been confirmed explicitly. In our clinic cases of RRP were found in children born by caesarean section. In the majority of individuals most HPV infections remain transient and asymptomatic. In most cases, Human Papillomavirus infection resolves within 2–4 years [12].

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Objective. The main purpose of this study was to establish the level of Human Papillomaviruses carrier state in the upper respiratory tract mucosa of healthy pre-school children. The second aim was to identify potential risk factors for HPV infection.

MATERIALS AND METHOD

After obtaining consent from their parents, 97 pre-school children were examined – 51 girls (52.6%) and 46 boys (47.4%), aged between 3 – 5 years; average age – 4 years and 5 months. 68 children (70.1%) were urban dwellers and 29 (29.9%) originated from a rural environment. A questionnaire with detailed history was taken which included personal details of both parents and children, as well as perinatal risks factors in pregnancy. Questions concerning the general health of a child were asked and sensitivity to upper respiratory tract and skin infections. Socio-demographic information was also obtained, including the standard of living and chosen environmental factors. Routine ENT examination was performed. Exfoliated oral squamous cells were collected from swabs and analysed for the presence of DNA papillomaviruses by polymerase chain reaction. DNA from oral squamous cells was isolated by proteinase K digestion (proteinase K 50μg/ml, SDS 0.5%, Tris HCl 50mM pH 7.5, EDTA 5mM pH 8.0, NaCl 50mM) for 24 hrs at 37˚C and phenol-chloroform extraction performed as described by Fife et al. [13]. DNA was precipitated from supernatant in a sodium acetate ethanol solution, dissolved in 50μl of TE (Tris HCl 10mM pH 8.0, EDTA 1mM pH 8.0), and used for future study. PCR was performed. For amplification of the DNA, 4 primer pairs from the L1 region of HPV-16, E1 region of HPV-18, L1 region of 5 types of HPV, and the L1 region of 33 types of HPV were used. The sequences of primers are shown in Table 1.

Table 1. Oligonucleotide primers used in PCR

HPV type	Primer Sequence (5`-`)	Map location	Product size
HPV 16	GCCTGTGTAGGTGTTGAGGT TGGATTTACTGCAACATTGG	5939–5958 6165–6184	246bp
HPV 18	GTGGACCAGCAAATACAGGA TCCAACACGTGGTCGTTGCA	2361–2380 2503–2522	126bp
5 types of HPV	ACTGGCTTTGGTGCTATGGACTTT GTACTGCGTGTAGTATCAACAACAG	L1 region 5 types of HPV	413bp
33 types of HPV	CGTCCMARRGGAWACTGATC GCMCAGGGWCATAAYAATGG M=A+C, R=A+G, W=A+T, Y=C+T	L1 region 33 types of HPV	450bp

The amplified DNA was separated by electrophoresis on 1.5% agarose gel in 0.04M Tris-acetate (pH 8.0) and 0.001M EDTA at 100V and 10mA for 1 h. The gel was stained with ethidium bromide and visualized by UV transillumination. In some cases, extracted DNA was evaluated for HPV by DNA sequencing.

RESULTS

19 children (19.6%) in the group were found to be infected. The criterion of infection was the presence of DNA sequences characteristic for HPV 6 and/or 11 squamous cells taken from the buccal mucosa. Of these 19 children, 11 were girls and 8 boys; average age – 3 years and 10 months. The DNA sequence characteristic for 'high risk' was not detected in any of the analysed samples. Statistical analysis did not reveal significant differences between rural and urban dwellers.

Table 2. Residence factors

Gender	Study group – 97 (100%)		City dwellers – 68 (70.1%)		Village dwellers – 29 (29.9%)	
	HPV +	HPV -	HPV +	HPV -	HPV +	HPV -
Girls	11 (21.6%)	40 (78.4%)	8 (22.2%)	28 (77.8%)	3 (20%)	12 (80%)
Boys	8 (17.4%)	38 (82.6%)	6 (18.8%)	26 (81.2%)	2 (14.3%)	12 (85.7%)
TOTAL	19 (19.6%)	78 (80.4%)	14 (20.6%)	54 (79.4%)	5 (17.2%)	24 (82.8%)

Statistical analysis of the questionnaire data did not reveal any relationship between the presence of Human Papillomaviruses DNA and the course of pregnancy, diseases a mother suffered from during pregnancy, drug taking in pregnancy, or exposure to unfavourable environmental factors. No significant correlations were found between HPV infection in the upper respiratory tract mucosa and the type and time of delivery, or birth weight of the child.

Table 3. Perinatal factors

	Study group – 97 (100%)		City dwellers – 68 (70.1%)		Village dwellers – 29 (29.9%)	
	HPV +	*HPV -*	*HPV +*	*HPV -*	*HPV +*	*HPV -*
Premature birth	2	5	2	4	0	1
Caesarean section	3	8	2	6	1	2
Average birth weight	3,300	3,180	3,220	3,080	3,450	3,200

Information from the questionnaire revealed that the parents of 9 HPV positive children – 47.4% – were active smokers, while in the HPV negative group of children the percentage was 26.9 – 21 children. A fact worth considering is the standard of living, which in the families of HPV positive children was estimated by 42.1% (8 families) as low or rather low, while in the other group 23.1% (18 families) gave a similarly bad estimation.

Table 4. Environmental factors

	Study group – 97 (100%)		City dwellers – 68 (70.1%)		Village dwellers – 29 (29.9%)	
	HPV +	*HPV -*	*HPV +*	*HPV -*	*HPV +*	*HPV -*
Passive smoking	9 (47.4%)	21 (26.9%)	5 (35.7%)	11 (20.4%)	4 (80%)	10 (41.7%)
Low standard of living	8 (42.1%)	18 (23.1%)	4 (28.6%)	10 (18.5%)	4 (80%)	8 (33.3%)

DISCUSSION

In the literature, examination of tissue samples taken from patients with Recurrent Respiratory Papillomatosis (RRP), using the PCR method with specific primers, revealed HPV type 6 and/or 11 in 98%-100%. Viral DNA were also found in exfoliated mucosal cells from the nasal cavity, tonsils and

throat of ill children, as well as in blood samples of patients with a particularly active form of the disease (4 and more microsurgeries per year performed to relieve acute dispnoea due to the growth of papillomas).

The presence of HPV DNA in the upper respiratory tract mucosa of asymptomatic children was detected in wide range: between 1.9 – 41% of analysed samples [14]. In the presented study, 'low risk' HPV was detected in 19 children (19.6%). The DNA sequence characteristic for HPV-16 and HPV-18 was not identified in any of the analysed cases.

To discuss the obtained results appeared to be a very difficult task as only a few publications exist on asymptomatic HPV infections of the upper respiratory tract in children, and different methods were used for DNA identification. Detection of Human Papillomaviruses in swabs taken from healthy children and the carrier state of 19.6% suggest that respiratory tract infection due to HPV is a common phenomenon. HPV infection is the most common sexually-transmitted disease in humans, but the modes of viral transmission in children remain controversial and include perinatal transmission, auto- and hetero-inoculation, sexual abuse, and possibly, indirect transmission via contaminated objects. Iatrogenic infection during first manoeuvres in neonates should be considered [1, 9, 15, 16, 17].

Different types of mucosal HPV are often present in the genital tract which may result in infecting a newborn during its passage through the mother's birth canal, or perinatally. The incidence rate of HPV DNA presence in oral swabs of newborns and infants varies from 4% – 94%. The correlation of Papillomaviruses types presented in newborns and their mothers is in the range of 50% – 69% of cases [18].

Statistical analysis of the presented material shows the same incidence of the infection in girls and in boys. On average, the infected children were at the same age as those in whom viral DNA was not detected. The understanding of the possible viral transmission routes is important, in particular because several vaccination programmes are being planned worldwide.

The study did not show any relationship between the incidence of infection and risk factors of pregnancy, such as viral infections of a mother or drug taking. Moreover, the time and type of delivery and the condition of a baby at birth do not determine HPV infection. A significantly higher level of active smokers and families with a low standard of living may suggest a considerable contribution of environmental factors in the spread of HPV infection.

CONCLUSIONS

1. The presence of HPV in the respiratory tract of children is relatively high – 19.6%.
2. 'High oncogenic potential' HPVs, such as HPV-16 and HPV-18, were not observed in the squamous cell mucosa of the respiratory tract in the children examined.
3. There were no significant differences between HPV carrier state between urban and rural dwellers.

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