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Citation

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ORIGINAL ARTICLE

HPV type-specific distribution among family members and linen in households of cutaneous wart patients

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Abstract

Background Common and plantar warts are caused by human papillomaviruses (HPV). Mode of transmission of wart HPVs within families is largely unknown.

Objective To demonstrate similarity of HPV type(s) among wart cases, family members and household linen.

Methods In a cross-sectional study, swabs taken from 123 warts and foreheads of 62 index patients and 157 family members and from 58 kitchen towels and 59 bathroom mats were tested for DNA of 23 cutaneous wart-associated HPV types. Generalized estimating equations (GEE) were used to estimate the chance of detecting the same HPV type as was found in the index patients on the family contacts and on the kitchen towels and bathroom mats.

Results HPV1, HPV2, HPV27 and HPV57 were the most prevalent types in the warts of the index patients. Altogether, 60 (42.3%) of the 142 family members without warts had HPV DNA on their foreheads. When HPV1 and HPV2 were found in the warts, these types were also frequently (>50%) found on the foreheads of index patients and their family members, as well as on the kitchen towels and the bathroom mats. HPV27 and HPV57 were less frequently found (<25%) on foreheads and linen. No associations were found for age, sex and site of HPV DNA presence.

Conclusion Dissemination of skin wart-causing HPV types, from wart cases to household contacts and linen, such as kitchen towels and bathroom mats, is more likely for HPV1 and HPV2 than for HPV27 and HPV57. The role of towels and bathroom mats in HPV transmission deserves further investigation.

Received: 25 May 2021; Accepted: 16 September 2021

Conflict of interest None of the authors had any conflict of interest.

Funding sources There were no funding sources that supported this work.

Introduction

Cutaneous warts are a common viral infection in the general population, especially in children, and are caused by human papillomaviruses (HPV).1 One-third of primary school children have one or more warts on their hands or feet,2 with an incidence of 29 per 100 person-years for developing new warts.3

HPV types belonging to the alpha (HPV2, HPV3, HPV7, HPV10, HPV27, HPV28, HPV29, HPV40, HPV43, HPV57, HPV77, HPV85, HPV91 and HPV94), gamma (HPV4, HPV65, HPV95, HPV48, HPV50, HPV60 and HPV88), mu (HPV1 and HPV63) and nu genus (HPV41) are known to induce cutaneous warts.4 The most prevalent HPV types in cutaneous warts in the general population are HPV27 (24%), HPV57 (22%), HPV2 (22%) and HPV1 (19%).5 The relative contribution of these four HPV types combined is 86%.3
HPV can be transmitted by direct skin contact with infected skin.6 Multiple risk factors for the development of cutaneous warts have been proposed, such as floors of public showers, swimming pools, locker room environment, classrooms, family members with warts, pre-existing warts and gender. According to recent studies, environmental factors do not play such a significant role for the transmission of warts. Having family members with cutaneous warts has been shown to be a more important risk for developing warts.2,3

The HPV distribution of warts within families has not yet been investigated, and no data are available on HPV carriage and transmission within families. The goal of this cross-sectional study was to elucidate the distribution of HPV within households where at least one person (index patient) was diagnosed with one or more common and/or plantar warts. Swabs were taken from the foreheads (clinically normal skin) of the index patient and family members, to analyse the distribution among household members of the HPV types found on the warts of the index patient. Furthermore, we investigated the kitchen towels and bathroom mats for HPV type presence in the families. We hypothesize that these common shared items may function as the reservoir for the virus contributing in the transmission of cutaneous warts-associated HPV in families is. As these two objects are used by the entire family, we suspect common warts to be more likely transmitted through kitchen towels and plantar warts through bathroom mats.

**Patients and methods**

The current study was part of the WARTS-2 trial,7 which was a multicentre, randomized, parallel-group superiority trial to compare the effectiveness of monochloroacetic acid with the conventional treatments (cryotherapy and salicylic acid) against common and plantar warts. We used the inclusion criteria of the national treatments (cryotherapy and salicylic acid) against the effectiveness of monochloroacetic acid with the conventional treatments (cryotherapy and salicylic acid) against common and plantar warts. Swabs were taken from the foreheads (clinically normal skin) of the index patient and family members, to analyse the distribution among household members of the HPV types found on the warts of the index patient. Furthermore, we investigated the kitchen towels and bathroom mats for HPV type presence in the families. We hypothesize that these common shared items may function as the reservoir for the virus contributing in the transmission of cutaneous warts-associated HPV in families is. As these two objects are used by the entire family, we suspect common warts to be more likely transmitted through kitchen towels and plantar warts through bathroom mats.

The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Centre (number P09.097) and registered in the Dutch trial registration: NTR1771.

Participants were also asked to fill in questionnaires regarding the following characteristics: sex (male vs. female), age, number of residents within a family and location of warts (hands, feet and other locations).

All tubes were sent to DDL Diagnostic Laboratory in Rijswijk, the Netherlands, for HPV genotyping. The HSL-PCR/MPG assay (DDL Diagnostic Laboratory, Rijswijk, the Netherlands) was used for genotyping all known wart-associated HPVs from the alpha genus (species 2: HPV3, 10, 28, 29, 77, 94; species 4: HPV 2, 27, 57; species 7: HPV85; species 8: HPV7, 40, 43, 91), the gamma genus (species 1: HPV4,65,95; species 2: HPV50; species 3: HPV48; species 4: HPV60; species 5: HPV88), the mu genus (species 1: HPV1; species 2: HPV63) and the nu genus (HPV41). This assay has been evaluated and described in detail by de Koning et al.4 A cut-off of 200 median fluorescence intensity (MFI) readouts was used to dichotomize between present and absent HPV DNA.

Logistic regression was used to analyse the association between HPV presence in one or more warts in the index patients with the HPV presence on the foreheads of the index patients or one or more family members and the kitchen towels and bathroom mats.

Generalized estimating equations (GEE) were used to account for the dependence between members of the same family, allowing the odds ratio (OR) to be calculated for the presence of HPV on foreheads of family members, with valid 95% confidence interval (CI) while making use of all warts in the index patients and all foreheads in the family members. GEE is an extension of the generalized linear model (GLM) and is used when the outcome variable is dichotomous and the responses are correlated. While the responses between the families (clusters) are uncorrelated and taken independently, the within-family responses are correlated and dependent. To account for this variation within and between clusters the effective sample size is not the number of warts, but number...
of clusters (families). Ignoring this correlation structure can affect the standard error. After executing the model in SPSS, a quasi-complete separation was detected for some items. Quasi-complete separation happens when the outcome variable separates a predictor variable or a combination of predictor variables to a certain degree. In case independent variables, e.g., presence of the HPV1 on the kitchen towel, were perfectly separated the outcome variable (presence of the same HPV DNA on the warts of index patient) we randomly changed one of the HPV1-negative kitchen towel samples to HPV1 positive, preventing an infinite risk estimate and allowing to generate ORs with 95% CI.

Table 1 Baseline characteristics of the index patients according to the most frequently occurring HPV types

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Index patient</th>
<th>Warts</th>
<th>Forehead swabs</th>
<th>Family members</th>
<th>Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Persons (N = 62)</td>
<td>Warts (N = 123)</td>
<td>Index pat* (N = 62)</td>
<td>All (N = 157)</td>
<td>Kitchen towel† (N = 58)</td>
</tr>
<tr>
<td>1 (mu)</td>
<td>17 (27.4)</td>
<td>24 (19.5)</td>
<td>16 (25.8)</td>
<td>18 (29.0)</td>
<td>38 (30.9)</td>
</tr>
<tr>
<td>2 (alpha)</td>
<td>10 (16.1)</td>
<td>20 (16.3)</td>
<td>7 (11.3)</td>
<td>8 (12.9)</td>
<td>13 (10.6)</td>
</tr>
<tr>
<td>27 (alpha)</td>
<td>19 (30.6)</td>
<td>34 (27.6)</td>
<td>6 (9.7)</td>
<td>7 (11.3)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>57 (alpha)</td>
<td>16 (25.8)</td>
<td>30 (24.4)</td>
<td>2 (3.2)</td>
<td>2 (3.2)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Gamma†</td>
<td>7 (11.3)</td>
<td>10 (8.1)</td>
<td>4 (6.5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rest**</td>
<td>4 (6.5)</td>
<td>4 (3.3)</td>
<td>1 (1.6)</td>
<td>4 (6.5)</td>
<td>4 (6.5)</td>
</tr>
<tr>
<td>Total††</td>
<td>59 (95.2)</td>
<td>113 (91.7)</td>
<td>29 (46.8)</td>
<td>35 (56.5)</td>
<td>64 (40.8)</td>
</tr>
</tbody>
</table>

HPV3, 28, 29, 77, 94, 85, 7, 40, 43, 91, 48 and 63 were not detected in any sample.

*The upper numbers show HPV-positive forehead swabs in all index patients, the lower number in index patients with the same HPV type in their warts.
†The upper numbers show HPV-positive forehead swabs in at least one family member, the lower number in at least one family member if the index patients had the same HPV type in their warts.
‡The upper numbers show all HPV-positive kitchen towels, the lower number HPV-positive kitchen towels if the index patients had the same HPV type in their warts.
§The upper numbers show all HPV-positive bathroom mats, the lower number HPV-positive bathroom mats if the index patients had the same HPV type in their warts.
†Gamma HPV types 4, 50, 60, 65, 88, 95.
**Alpha HPV type 10 and nu HPV type 41.
††Some samples contained multiple HPV types.
Results

From 62 families (62 index patients and 157 family members) a total of 476 swab samples were collected. Nine samples of warts from family members, four samples from kitchen towels and three from bathroom mats were not collected or lost during transport to the laboratory, resulting in a final collection of 123 wart samples from 62 index patients and 7 warts from 7 family members, 62 forehead samples of index patients and 157 forehead samples of family members, 58 kitchen towel samples and 59 bathroom floor samples.

A summary of the baseline characteristics of the 62 index patients according to the most frequently occurring HPV types is presented in Table 1. These data are also provided per family in the supplementary table that provides the HPV distribution among index patients and family members for all families, separately. In 8 of the 123 warts, multiple HPV types were detected (1 + 27; 2 + 4; 2 + 27; 2 + 41; 27 + 57; 27 + 57; 41 + 57; 1 + 4 + 41 + 65) and sometimes different warts in the same index patients harboured different HPV types (see Table S1). Fifteen of the 157 family members had warts (2 were HPV27 positive, 5 were HPV negative and 8 samples were lost). Altogether, 60 (42.3%) of the 142 family members without warts had HPV DNA on their foreheads.

Patients with HPV1 in their warts were significantly younger and more often had plantar warts, compared with patients with HPV2-, HPV27- or HPV57-positive warts. The median age of the 148 family members (9 missing values) was 35.0 years (quartiles 16.0; 43.0) and half of them (74; 50.0%) were men. Patients with HPV1 in their warts were significantly younger and more often had plantar warts, compared with patients with HPV2-, HPV27- or HPV57-positive warts. The median age of the 148 family members (9 missing values) was 35.0 years (quartiles 16.0; 43.0) and half of them (74; 50.0%) were men.

The distribution of the most frequently detected HPV types among warts, foreheads, kitchen towels and bathroom mats in index patients and family members is summarized in Table 2. The most frequently detected types in the warts of the index patients were HPV2 (16.1%), HPV27 (30.6%) and HPV57 (25.8%) belonging to the alpha papillomavirus genus, species 4 and HPV1 (27.4%) from the mu-papillomavirus genus, species 1 (Tables 1 and 2).

The spread of the HPV types appeared to be different for HPV1 and HPV2 on one side and HPV27 and HPV57 on the other side (Tables 2 and 3–6). When HPV1 and HPV2 and
gamma HPV were detected in warts of the index patients, there was a high presence (around 70%) of these types in forehead swabs of the index patients and family members and in swabs from the kitchen towels (around 45%) and the bathroom mats (around 60%). HPV27 and HPV57 were much less frequently detected (less than 25%) in the surroundings than HPV1 and HPV2 (Tables 2 and 3). Subgroup analyses for common and plantar warts, separately, showed no important difference in the presence of the same HPV type in one or more warts of the index patients and family members and in swabs of kitchen towels and bathroom mats. Gamma HPV types 4, 50, 60, 65, 88, 95.

Table 5: The chance of having HPV DNA in swabs of the kitchen towel in the presence of the same HPV type in one or more warts of the index patients

<table>
<thead>
<tr>
<th>HPV type in one or more warts</th>
<th>HPV in swabs kitchen towel</th>
<th>GEE on 123 warts and family members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent N(%)</td>
<td>Present N(%)</td>
<td>Logistic regression in 62 index patients</td>
</tr>
<tr>
<td>1 (mu)</td>
<td>45 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>9 (52.9)</td>
<td>8 (47.1)</td>
</tr>
<tr>
<td>2 (alpha)</td>
<td>52 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>6 (60.0)</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td>27 (alpha)</td>
<td>43 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>18 (94.7)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>57 (alpha)</td>
<td>46 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>13 (81.3)</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Gamma†</td>
<td>52 (94.5)</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>Present</td>
<td>85.7</td>
<td>1 (14.3)</td>
</tr>
</tbody>
</table>

*The upper odds ratios show the non-adjusted odds ratios, and the lower odds ratios are adjusted for age and sex of the index patients and the location of the warts.
†Gamma HPV types 4, 50, 60, 65, 88, 95.

Discussion

This is the first study to investigate the transmission of cutaneous wart-associated HPV types within families. HPV1 and HPV2 detected on the warts of the index patients were also detected on the foreheads of the index patients and family members, as well as on the kitchen towels and bathroom mats. We cannot tell the sequence of these occurrences, but it is likely that the foreheads, kitchen towels and bathroom mats were contaminated with HPV originating from the ‘index’ wart. Whether the linen served as an important intermediate in the spread of these HPV types within these families remains to be seen. We were not able to prove that common warts are more likely to spread via the kitchen towels and the plantar warts via the bathroom mats, possibly because of overlap between patients with common and plantar warts (14.8% of the patients) or the low frequency of HPV-positive linen. The spread of HPV27 and
HPV57 was much more modest within the families. It is not likely that the spread via the kitchen towels and bathroom mats played an important role for HPV27 and HPV57.

While HPV1 (mu papillomavirus genus, species 1) and HPV2 (alpha papillomavirus genus, species 4) are classified in different genera, they showed a similar distribution pattern. HPV1-induced warts present with a distinct clinical profile. HPV1 is most often found on plantar location in children aged <12 years with a duration of <6 months and are often resolved before seeking medical care. HPV2 and also HPV27 and HPV57 are more common in people ≥12 years, and HPV2 is more often located on the hands.

Most HPV types share a similar life cycle consisting of an early and a late phase. The early phase consists of infecting stem cells in the basal layer of the skin to form a reservoir of low productivity of the viral genome (50–100 copies of viral genome per cell). Once the infected cells migrate to the upper layers of the skin, viral genome amplification is started by the expression of high levels of replication proteins (E1, E2, E4 and E5) producing thousands of copies of viral genome per cell and the development of warts. Lastly, infectious virions are released into the environment to infect a new host. It is proposed that the release of infectious virions from the skin is supported by the protein E4 which disrupts the keratin structure of the skin. The high dispersal of HPV1 on the clinically normal skin and the linen could be explained by its high viral particle synthesis. Compared to other HPV types, HPV1 genome amplification starts immediately in parabasal cell layers of the skin and the viral protein E4, important for genome amplification efficiency and virus synthesis, is highly abundant in HPV1, leading to high viral particle synthesis. Possibly HPV2 also behaves like HPV1 leading to high viral particle synthesis and its abundance on non-wart samples. The discrepancy in distribution, clinical characteristics and likely transmission between these HPV types needs to be investigated in future studies.

Although HPV1 and HPV2 were the most common types on the forehead of family members, they only occasionally led to the development of warts in family members in our study. This finding questions the meaning of the presence of HPV DNA on patients skin, as 42.3% of the patients without plantar warts carried HPV DNA. The low frequency of HPV1- and HPV2-induced warts in family members with prevalent carriage may reflect the low virulent abilities of these HPV types, as more of these virus particles may be needed to develop warts compared to other HPV types. Whether or not these patients are at risk for developing warts themselves or are a risk to the population needs to be further investigated, for instance in a cohort, follow-up study.

The study group consisted of patients visiting their general practitioner, therefore better reflecting the general population than patients from a dermatology department. In accordance with previous studies, the high prevalence of HPV1, HPV2, HPV27 and HPV57 was supported in this study. Recently, in a study investigating HPV distribution in elementary school classes, it was shown that HPV1 was detected in only two warts (3%). We detected HPV1 in 24 (19.5%) warts, which difference could be due to possible selection bias in the present study, or the use of a different, more sensitive PCR. We investigated patients who visited their general practitioner for their cutaneous warts. HPV1 tends to cause deep and painful warts leading to more frequent visits to the general practitioner which may have led to a larger proportion of people with HPV1-positive warts.

A possible limitation of the study is that although the clinical investigation was performed by trained research nurses, the skin was not evaluated by a dermatologist. In addition, we tested only swabs from the surface of the respective warts, but in an earlier study we had shown that HPV types of cutaneous warts can be reliably identified by surface swabs. Another limitation of this study is the small sample collected from the warts of family members. It would be interesting to know whether the same HPV DNA could be detected in families with multiple family members with warts. The number of tested wart samples from family members was too small (n = 7) for statistical analysis. In five warts of family members no HPV DNA was found and the remaining 2 warts were caused by HPV27. In both families there was an agreement with the warts of the index patient. The cross-sectional design prohibits test for causal relationships and modes of transmission. Prospective studies are needed to analyse the risk of transmission and of the development of warts in families with HPV-positive index patients.

In summary, HPV1, HPV2, HPV27 and HPV57 appeared to be the most important pathogens for the spread of cutaneous warts within families. Kitchen towels and bathroom mats may be important reservoirs possibly contributing to the spread of HPV1 and HPV2 in families.

References
7. Bruggink SC, Gusekloo J, Egberts PF et al. Monochloroacetic acid application is an effective alternative to cryotherapy for common and planar wart.

Supporting information
Additional Supporting Information may be found in the online version of this article:
Table S1. HPV genotype detection in 62 families