Worldwide Prevalence of Human Papillomavirus in Tonsillar Squamous Cell Carcinoma and Tumor-Free Tonsillar Tissue

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Abstract

Human Papillomavirus (HPV) is the etiological agent in cervical cancer and a significant proportion of anogenital cancers. It appears to also be associated with a subset of head and neck cancers, in particular tonsillar squamous cell carcinoma (TSCC). The overall prevalence of HPV and the type-specific prevalence at the cervical level vary geographically. It is unclear whether this is also the case in tonsillar infection with HPV and in HPV-associated TSCC. This review provides an overview of the current literature with regards to the prevalence and types of HPV found in TSCC, and in tumor-free tonsillar tissue, globally.

The HPV prevalence in TSCC is highly variable from region to region, ranging from 0 to 100%. The majority of studies from Europe, however, report an HPV prevalence of 40-60%. A similar prevalence of 35-65% is reported in North America. The only study from Australia reports a comparable prevalence of 46%. In contrast, the prevalence in China and Taiwan is between 0 and 13%. No data is available from Latin America, Africa, Eastern Europe, and other parts of Asia. The predominant type identified is HPV 16, with the majority of studies detecting HPV 16 in over 80% of HPV-positive samples. HPV 33 is the second most frequent type. Other high-risk and low-risk types are only rarely isolated. Co-infections with multiple types are also rare. Several Western European countries, USA and Australia, have reported an increase in the incidence of TSCC cases. There is evidence that this is attributable to an increase in HPV-associated TSCC cases. In tumor-free tissue the overall HPV prevalence ranges between 0 and 9%, and the most frequent type identified is HPV 16. HPV 11 has also occasionally been isolated. The interpretation of these results is complicated by the small sample size of many studies and by heterogeneous methodology.

Keywords: Human Papillomavirus; Tonsil; Head and neck squamous cell carcinoma; Epidemiology

Abbreviations: HPV: Human Papillomavirus; TSCC: Tonsillar Squamous Cell Carcinoma; HNSSC: Head and Neck Squamous Cell Carcinoma

Introduction

Head and neck squamous cell carcinoma (HNSSC) encompasses a multitude of cancer types, arising from the mucosal lining of the oral cavity, oropharynx, hypopharynx, larynx, sinonasal tract, and nasopharynx. Alcohol and tobacco are well-recognized pre-disposing factors for these cancers. Several Nordic countries (Denmark [1], Sweden [2][3], Finland [4]), the United States [5-8] and Australia [9], have reported an increase in the incidence of tonsillar and base of tongue carcinoma, despite a decrease in alcohol and tobacco consumption in these countries and despite a decrease in other HNSSC subtypes. Interestingly, this increase has been primarily in younger males (under 60 years) with no history of excessive alcohol and tobacco use [1,10]. These cancers appear to have not only a different clinical profile, but also a different histological and molecular profile, and a significantly better prognosis independent of stage at diagnosis, compared to those induced by excessive tobacco/alcohol consumption [11]. Large epidemiological studies suggested a link between tonsillar carcinoma and Human Papillomavirus (HPV), the etiological agent in cervical cancer and in a significant proportion of anogenital cancers. These studies showed that patients with an HPV-associated anogenital cancer have a 4-fold increased risk of tonsillar squamous-cell carcinoma [12] compared to patients with a non-HPV-associated cancer type, and that in fact husbands of patients with cervical cancer also developed an excess of both tonsillar cancer and cancer of the tongue [13]. Over the last two decades significant evidence has accumulated that further strongly supports that HPV is associated with this subset of head and neck cancers [14-17]. Recently, Hammarstedt et al. [2] and Näsman et al. [3] have shown that the increase in tonsillar carcinoma in Sweden coincides with an increase in the number of Human Papillomavirus (HPV)-positive cases (remarkably, HPV-associated cases rose from 23% in the 1970s to 93% in 2006 [3]), leading them to suggest that we are dealing with a viral-induced cancer epidemic.

Over the past two decades multiple epidemiological studies have aimed to characterize the epidemiology of HNSSC, and in particular the prevalence of HPV in these cancers. The results of these studies are drastically divergent [10], with the proportion of HPV-associated cases ranging anywhere from 0 % to over 90% [10,18,19]. Many of the studies report on HNSSC cases overall, while others categorize results into a few anatomical subgroups (i.e., oropharyngeal vs. oral cavity vs. hypopharynx/larynx), and only a few studies report on the epidemiology of tonsillar carcinoma and base of tongue cancer specifically. Furthermore, some studies classify the base of the tongue as part of the oral cavity, while others classify it as part of the oropharynx. Some authors use the word tonsils to refer solely to the palatine tonsils, while others include the lingual tonsils in this term. This variability...
in classification and reporting makes it very challenging to obtain an overview of the epidemiology of tonsillar carcinoma globally, as well as of the proportion of HPV-associated cases.

This review aims to provide a brief overview of the epidemiology of tonsillar squamous cell carcinoma (TSCC) worldwide, based on the current literature. An extensive review regarding HPV tonsillar infections and tonsillar carcinoma has previously been provided by Dr. Syrjänen in 2004 [4]. The present review focuses on the palatine tonsil only. Data regarding base of tongue carcinoma and carcinoma of other Waldeyer’s ring structures is not covered here, as the data available at the moment is limited. Given that the association with HPV is strongest for tonsillar and base of tongue carcinoma, and that HPV-positivity appears to have prognostic significance, it is essential to consider these specific anatomic sites separately, both for research and clinical purposes. It is further relevant to define the epidemiology of these carcinomas at the outset of HPV-vaccination, to evaluate the potential impact of vaccination in the prevention of these cancers.

Human Papillomavirus – A Brief Overview

Human Papillomavirus (HPV) belongs to the Papillomaviridae family. It is a small double-stranded, circular DNA virus, which infects epithelial tissue and depends on epithelial differentiation for completion of its lifecycle. More than 100 HPV types have been identified, and they have been subdivided according to their malignant potential into high-risk and low-risk. They can also be categorized as mucosal or cutaneous, based on the site they infect. The high-risk types (e.g., 16 and 18) are associated with cervical cancer and anogenital cancers. The low-risk types (e.g., 6 and 11) are associated with benign neoplasms, such as cutaneous and genital warts, as well as oral papillomas and recurrent respiratory papillomatosis [20].

HPV has been extensively studied in the context of cervical cancer, as there is a strong causal relationship, with the virus being found in 99.7% of such cancers [21]. The two most frequent oncogenic subtypes in cervical cancer are 16 and 18. A smaller, but significant, proportion of anogenital cancers are associated with HPV (90% of anal cancers, 60-90% of vaginal and vulvar cancers, and 30-40% of penile cancers) [22]. The predominant type in these cancers is type 16.

Epidemiological studies on HPV cervical infection and cervical cancer have demonstrated that the overall HPV prevalence and type-specific distribution vary worldwide. For example, HPV prevalence in women with normal cervical cytology varies from 1.7% in Western Asia to over 30% in the Caribbean and Sub-Saharan Africa [23]. HPV 16 is the predominant type worldwide, however, the proportion of type 16 infections varies by region. The prevalence of HPV 16 infections is higher in North America and Europe, and lower in Africa, Asia and Latin America. The next most frequent types vary from region to region [23]. Whether such heterogeneity is also present in TSCC is unclear.

Overview of HPV Prevalence in HNSCC

The incidence of HNSCC varies from country to country [24]. Population-based differences in the rate of tobacco smoking/chewing [25], betel nut chewing [26], and alcohol consumption [25] are in part responsible for this variability. Other contributing factors may be poor oral hygiene [27] and dietary factors [28,29] although these are less well-established risk factors. Furthermore, for HPV-associated cases certain sexual behaviours [27,30] seem to be additional risk factors, and may be more prominent in certain regions. Genetics likely play an additional role (i.e., differences in DNA repair capacity, increased sensitivity to carcinogens, immune system deficiencies), however, the specific genetic mechanisms remain to be determined.

Kreimer et al. [31] performed a systematic review to determine the worldwide prevalence and type distribution of HPV in over 5000 HNSCC tumor biopsies. Data was extracted from published studies that used clearly described PCR detection methods. The prevalence of HPV in squamous cell carcinoma (SCC) of the oropharynx was found to be 28.2% in Europe (primarily based on studies from Western Europe), 37.0% in North America, and 46.3% in Asia (Japan, India). There were only a few studies from Australia, Central/South America, and Africa. Therefore, results of studies from these regions were pooled together, for a prevalence of 36.6% in this group. The overall prevalence was lower in oral SCC (23.5%) and hypopharynx/larynx (24.0%) vs. oropharyngeal SCC (35.6%). HPV 16 was consistently the predominant type detected. Kreimer et al. [31] note that these results must be interpreted with caution as cases may have been misclassified at the time of diagnosis (i.e., an advanced oropharyngeal SCC as an oral SCC), and that further discrepancies could arise due to the small sample size of some studies (i.e., selection bias, publication bias). Of further note, the review classified tongue cancers as oral cavity cancers. However, the base of the tongue is often classified anatomically as an oropharyngeal site. Hence, the proportion of HPV-positive oropharyngeal cases may be underestimated, and conversely those of the oral cavity overestimated. The variable HPV prevalence across the anatomic sites considered further highlights the importance of reporting site-specific data.

Worldwide HPV Prevalence in TSCC

Table 1 shows the overall HPV prevalence and type-specific prevalence in TSCC by geographic region and by study. Studies with less than 10 patients have not been included. Only studies using HPV-detection methods were included (i.e., studies based only on seroprevalence to HPV were not included). A total of 19 studies were identified, 10 of which were from Europe (all from Western Europe) [3,14,19,32-40], six from North America (primarily USA) [41-45], two from Asia (Taiwan, China) [18,19], and one from Australia [19]. The results have not been pooled as different methods are employed in different studies. Paraffin-embedded tissue was used in 13 studies, fresh-frozen tissue was used in five, and brush samples of the oral cavity in combination with gargoyle specimens were used in one study. All studies verified the DNA quality of specimens by amplifying a human gene marker (i.e., beta-globin).

Overall HPV prevalence was drastically variable, with a range between 0 and 100% (Table 1). However, the majority of the studies (13/19) reported a prevalence of 40 to 65%. Results from Europe, North America and Australia largely indicated a prevalence consistent with this range; seven of the ten studies from Europe reported a prevalence between 40 and 60%, five of the six studies from North America reported a prevalence between 40 and 65%, and the only study from Australia reported a prevalence of 46%. Asia had a low prevalence (0-13%), although only two studies were available from this region, one of which had a small sample size.

HPV type-specific prevalence in TSCC

HPV16 is predominant in all studies analyzing HPV prevalence in TSCC. Eleven of the 14 studies reported an HPV16 prevalence of over 85% (of all HPV-positive samples). The remaining three studies report an HPV16 prevalence of 40%, 71%, and 73%. Other mucosal types identified, although rare, are HPV 18, 31, 33, 35, 39, 45, 51, 52, 59, and 67. In the European studies, HPV 33 is the second most frequently identified type, a finding not observed in the other regions. Several
<table>
<thead>
<tr>
<th>Study, Year</th>
<th>Country</th>
<th>Sample</th>
<th>Cases (n)</th>
<th>Overall and type-specific prevalence, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Any HPV</td>
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<tr>
<td><strong>Europe</strong></td>
<td></td>
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<td></td>
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<tr>
<td>St Guily et al., 2011 [32]</td>
<td>France</td>
<td>PE</td>
<td>185</td>
<td>106 (87%)</td>
</tr>
<tr>
<td>Harnisdal et al., 2010 [33]</td>
<td>Norway</td>
<td>PE</td>
<td>137</td>
<td>71 (52%)</td>
</tr>
<tr>
<td>Nåsman et al., 2009 [3]</td>
<td>Sweden</td>
<td>PE</td>
<td>98</td>
<td>83 (85%)</td>
</tr>
<tr>
<td>Hammersleddt et al., 2006 [34]</td>
<td>Sweden</td>
<td>PE</td>
<td>203</td>
<td>99 (49%)</td>
</tr>
<tr>
<td>Mellin et al., 2002 [40]</td>
<td>Sweden</td>
<td>FF</td>
<td>22</td>
<td>12 (55%)</td>
</tr>
<tr>
<td>Kussmann et al., 2001 [36]</td>
<td>Germany</td>
<td>PE</td>
<td>24</td>
<td>14 (58%)</td>
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<tr>
<td>Mellin et al., 2000 [35]</td>
<td>Sweden</td>
<td>PE</td>
<td>60</td>
<td>26 (43%)</td>
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<tr>
<td>Andl et al., 1998 [37]</td>
<td>Germany</td>
<td>FF</td>
<td>21</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>Snijders et al., 1992 [38]</td>
<td>Holland</td>
<td>FF</td>
<td>10</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Niedobitek et al., 1990 [39]</td>
<td>England</td>
<td>PE</td>
<td>28</td>
<td>6 (21%)</td>
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<tr>
<td><strong>North America</strong></td>
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<td></td>
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<tr>
<td>Luginbuhl, et al., 2009 [41]</td>
<td>USA</td>
<td>PE</td>
<td>48</td>
<td>17 (35%)</td>
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<tr>
<td>Pintos et al., 2008 [14]</td>
<td>Canada</td>
<td>Brush/ gargle</td>
<td>21</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Ernster et al., 2007 [42]</td>
<td>USA</td>
<td>PE</td>
<td>34</td>
<td>22 (65%)</td>
</tr>
<tr>
<td>Strome et al., 2002 [43]</td>
<td>USA</td>
<td>PE</td>
<td>52</td>
<td>24 (46%)</td>
</tr>
<tr>
<td>Ringström et al., 2002 [44]</td>
<td>USA</td>
<td>FF</td>
<td>11</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>Paz et al., 1997 [45]</td>
<td>USA</td>
<td>FF</td>
<td>15</td>
<td>9 (60%)</td>
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<tr>
<td><strong>Asia</strong></td>
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<tr>
<td>Chien et al., 2008 [18]</td>
<td>Taiwan</td>
<td>PE</td>
<td>111</td>
<td>14 (13%)</td>
</tr>
<tr>
<td>Li et al., 2003 [19]</td>
<td>China</td>
<td>PE</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td><strong>Oceania</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Li et al., 2003 [19]</td>
<td>Australia</td>
<td>PE</td>
<td>67</td>
<td>31 (46%)</td>
</tr>
</tbody>
</table>

1Three of the HPV-positive samples were not typable by sequencing.
2Seven of the HPV-positive samples were not typable by sequencing. Of further note, the HPV prevalence varied across time periods as follows: in 1970’s 7/30 (23%) were HPV-positive, in 1980’s 12/42 (29%), in 1990’s 48/84 (57%), and in 2000 to 2002, 32/47 (68%).
3HPV5b was isolated with HPV16, and in another case ADX1 with HPV16. HPV5b and ADX1 are cutaneous types.
4Brush sample obtained by swabbing multiple sites in the buccal cavity, was combined with a gargle specimen.
5Includes both palatine tonsil and back of tongue cases. No data given for palatine tonsil alone.
6Li et al. (AJP, 2003) have studied HPV prevalence in TSCC in China and in Australia. The same methods were used, except the FAP59/64 primer set was only used on the Chinese samples. Part of the Australian data is presented elsewhere (Li et al., Int J Cancer, 2003 and Li et al., Head and Neck, 2003).
7Three cases were positive for HPV by GP5+/6+ PCR but were not typable by sequencing. The authors suggest that multiple types or novel types were present in these samples.

Table 1: Overall and type-specific prevalence of HPV in tonsillar squamous cell carcinoma by geographic location and study. The type-specific prevalence represents types in either single or multiple infections. Only mucosal HPV types have been presented in the table. In certain cases the HPV type could not be identified. (PE = paraffin embedded, FF = fresh frozen).
studies have occasionally identified low-risk or intermediate-risk types [18,43], as well as cutaneous types, the significance of which is unclear. Infections with multiple HPV types are rare (generally <10%). In Taiwan [18] the proportion of HPV16-positive cases was lower (71%, 10/14), while HPV18 was found in 36% (5/14) of tumors. This study also found a higher prevalence of co-infections (29%).

**HPV Prevalence in Tumor-Free Tonsils**

Five relevant studies were identified in Western Europe [35,36,39,46,47], and five in North America [14,41,43,48,49]. Table 2 summarizes the results of these studies. HPV prevalence in tumor-free tonsils is reported between 0 and 9.4%. With regards to type, HPV 16 is the primary type detected. HPV 11 has also occasionally been detected. The age of the patients is also provided wherever available. This is relevant, as Chen et al. [47] have shown that the prevalence of HPV decreases with age after adolescence/young adulthood. This trend is also evident based on the results in Table 2, as studies on children report a higher HPV prevalence than studies on adults.

Table 3 provides an overview of the methods employed by the various studies for HPV detection in TSCC or tumor-free tonsils.

**Trends in TSCC**

Several studies have reported increases in the incidence of TSCC. In Denmark, this increase has been seen in both males and females, but most prominently in men younger than 60. The incidence rate among men rose from 0.63 (per 100,000 person-years) in 1978-1982, to 2.61 in 2003-2007, and among women from 0.25 to 0.88 [1]. Studies from other Nordic countries (Sweden [2], Norway [50], Finland [4]) have reported comparable results. Similar increases have been reported in Australia by Hocking et al. [9]. Consistent with the studies from the Nordic countries, the authors found that this increase was most marked within more recent birth cohorts (i.e., it peaked for cohorts born between 1945 and 1955). Frisch et al. [5] considered the incidence of TSCC over an extended period, from 1945 to 1995, in Connecticut, USA. They found that the incidence of TSCC increased fourfold among white women (likely secondary to an increase in smoking prevalence), but remained overall constant in white men over this period (complete data not available for non-white persons for the whole period). However, significant annual increases in TSCC were noted among men younger than 60 years between 1973 and 1995 (2.7% in blacks and 1.9% in whites). Shibosky et al. [6] have similarly reported an increase among younger white men in the USA between 1973 and 2001, but not amongst blacks. Ryerson et al. [8] have considered the incidence in the USA in recent years (1998 to 2003), and observed that the incidence of TSCC has continued to increase.

Some studies have considered HPV prevalence over time in TSCC. A study from Norway reported that 38% of samples, from 1960 to 1984, were positive for HPV. This proportion increased to 64% between 1985 and 1996 [33]. In Sweden, Näsman et al. [3] reported an HPV prevalence of 85% in TSCC between 2003 and 2007. These results were compared to a previous study, analyzing samples from 1970 to 2002, in the same region and employing the same methodology [2]. The prevalence of HPV-positive tonsillar cancer cases was 23% in the 1970s, 29% in the 1980s, 57% in the 1990s and 79% for 2000-2007. When considering 2006-2007 alone, the prevalence was up to 93%. In contrast, St. Guily et al. [32] did not find a trend with regards to HPV prevalence in France between 2000 and 2009.

Interestingly, the incidences of HPV-associated anal cancer and vulvar cancer have also been increasing over the last few decades [51,52]. These diseases are, like HPV-positive TSCC, believed to be associated with high-risk sexual behaviours [51]. Cervical cancer has been decreasing in particular in the developing world, due to rigorous screening programs. To our knowledge, there is no data to suggest that cervical HPV infection is increasing.

<table>
<thead>
<tr>
<th>Study, Year</th>
<th>Country</th>
<th>Sample</th>
<th>Cases (n)</th>
<th>Patient Age Range, Mean or Median</th>
<th>Overall and type-specific prevalence, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marmas, 2006 [46]</td>
<td>Greece</td>
<td>PE</td>
<td>64</td>
<td>2-14, mean = 7.5</td>
<td>16 (9%)</td>
</tr>
<tr>
<td>Chen, 2005 [47]</td>
<td>Finland</td>
<td>FF</td>
<td>212</td>
<td>1-72, mean = 23.2</td>
<td>132 (6%)</td>
</tr>
<tr>
<td>Klussmann, 2001 [36]</td>
<td>Germany</td>
<td>PE</td>
<td>14*</td>
<td>20-65, median = 52</td>
<td>0</td>
</tr>
<tr>
<td>Melin, 2000 [40]</td>
<td>Sweden</td>
<td>PE</td>
<td>10</td>
<td>18-48, mean = 27</td>
<td>0</td>
</tr>
<tr>
<td>Niedobitek, 1990 [39]</td>
<td>England</td>
<td>PE</td>
<td>30</td>
<td>47-80, median = 60</td>
<td>0</td>
</tr>
<tr>
<td>North America</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emster, 2009* [48]</td>
<td>USA</td>
<td>PE</td>
<td>226</td>
<td>21±</td>
<td>0</td>
</tr>
<tr>
<td>Luginbuhl, 2009 [41]</td>
<td>USA</td>
<td>PE</td>
<td>41</td>
<td>36-84*</td>
<td>0</td>
</tr>
<tr>
<td>Pintos, 2008 [14]</td>
<td>Canada</td>
<td>Brush/gargle*</td>
<td>129</td>
<td>25-84</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Sisk, 2006 [49]</td>
<td>USA</td>
<td>FF</td>
<td>50</td>
<td>3-12</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Strome, 2002 [43]</td>
<td>USA</td>
<td>PE</td>
<td>48</td>
<td>--</td>
<td>3 (6%)</td>
</tr>
</tbody>
</table>

*Study included both tonsillar and adenoid tissue, and authors did not state which subtypes were isolated from tonsillar tissue vs. adenoid tissue. Only the overall prevalence in tonsillar tissue was given. However, the authors state that primarily type 16 was isolated and less frequently HPV 11. No HPV 18 or 33 were isolated.

*Note that the study included patients across a broad age range. The prevalence was highest in preschool children (11.5%) and young adults (9.3%).

*Both tonsils tested for each patient in this study.

*Emster et al. have used the same technique in a series of oropharyngeal SCC and they detected HPV16 in 69% (50/72) [40].

*Ages of controls not given, however authors state that they were age-matched to the TSCC patients included.

*Brush sample obtained by swabbing multiple sites in the buccal cavity was combined with a gargle specimen.

Table 2: Overall and type-specific prevalence of HPV in tumor-free tonsils.
Study, Year | HPV-DNA detection and typing method
---|---
**Europe**
Hannisdal, 2010 [33] | GS5+/GS6+ touchdown PCR, sequencing
Näsmann, 2009 [3] | GS5+/6+ and CPI/IIG followed by sequencing, negative samples analyzed using HPV16 type-specific primers
Hammarstedt, 2006 [34] | GS5+/6+, CPI/IIG, negative samples analyzed using HPV 16 type-specific primers, samples positive by GS5+/6+ or CPI/IIG were sequenced for type
Mammas, 2006 [46] | GS5+/6+, then type-specific PCR for 16/18/33/11
Chen, 2005 [47] | Nested PCR with MY09/11 & GS5+/6+, genotype by sequencing
Mellin, 2002 [40] | GS5+/6+, negative samples were run with CPI/IIG, positive samples sequenced for genotype
Klussmann, 2001 [36] | Nested PCR with A10/A5-A6/A8, and CP62/70-CP65/69a, HPV16 type-specific PCR, typing by sequencing
Mellin, 2000 [35] | GS5+/GS6+, positive samples re-run with HPV16/33 type-specific primers
Andl, 1998 [37] | Type-specific primers for 6/11/16/18 followed by SB, negative samples rerun using consensus primers, products sequenced for genotyping
Snijders, 1992 [38] | GS5+/GS6+ followed by sequencing, type-specific PCR for HPV 16 and 33
Niedobitek, 1990 [39] | ISH with probes for HPV16, and HPV 6 and 11
**North America**
Ernster, 2009 [48] | Type-specific primers for HPV16 and 18
Luginbuhl, 2009 [41] | ISH with HPV III family 16B probe (probe cocktail for detection of high risk HPVs)
Pintos, 2008 [14] | PGM09/11, line blot assay for 27 HPV types
Ernster, 2007 [42] | Type-specific primers for HPV16 and 18
Siak, 2006 [49] | PGM09/11, Roche Linear Array for genotyping
Strome, 2002 [43] | MY09/11 followed by nested PCR for HPV-negative samples using primers 6582-23D and 7033-22 U, genotyping by sequencing; samples also run with HPV 16 type-specific primers, followed by SB for HPV16-negative samples
Ringström, 2002 [44] | MY09/11, typing by restriction enzyme digestion to detect HPV16 and 18
Paz, 1997 [45] | MY09/11 and IU/IWDO primers, samples positive by either primer set were analyzed using type-specific primers for HPV6, 16, and 18, samples also analyzed by SB
**Asia**
Chien, 2008 [18] | MY09/11, HPV positive samples analyzed by ISH for HPV6, 11, 16, 18, samples negative by ISH analyzed by linear array genotyping test (Roche)
**Oceania**

Table 3: HPV DNA detection and genotyping methods used in the studies presented in Table 1 and 2. (SB=Southern Blotting, ISH=In-situ hybridization).

**Discussion**

Differences observed in HPV prevalence between regions could arise from the heterogeneity of methods used. The majority of studies use sensitive PCR-based methods. However, different combinations of consensus primer sets and/or type-specific primer sets are used, and some studies use only one primer set, while others use multiple primer sets. Studies using only one primer set may underestimate the HPV prevalence. This is because consensus primers, which are able to detect a broad range of HPV types, amplify regions of the L1 gene (e.g., GS5+/6+, PGM09/11 primers) or the E1 region (e.g., CPI/IIG), however these genes are occasionally lost during the integration of viral DNA into host genomic DNA [53]. E6 and E7, however, are believed to be retained, and type-specific primers exist which amplify these regions. The E6 and E7 regions are too variable to develop consensus primers. It has been suggested that a combination of multiple consensus primers and type-specific primers should be used for optimal HPV detection [54]. Some studies did report increased detection through the use of multiple primer sets. For instance, Näsmann et al. [3] used two consensus primer sets (GS5+/GS6+ and CPI/IIG) for initial HPV-detection, and re-analyzed HPV-negative samples using HPV 16 E6-specific primers. 75 of 98 samples were positive using consensus primers, and eight additional samples were positive using the type-specific primers. Other studies use nested PCR, which is a very sensitive technique, able to detect low viral load [55,56]. Niedobitek et al. [39] and Luginbuhl et al. [41], which report the lowest prevalence amongst the European studies and the North American studies, respectively, both use in-situ hybridization with probes for various HPV types. This technique is not as sensitive as the PCR-based techniques used in other studies, and may explain the lower prevalence in these studies. Further heterogeneity is found at the genotyping level. The majority of studies used sequencing or line blot assays. One limitation of sequencing is difficulty in detection of multiple HPV types in the same sample. There is a lack of consensus on the optimal approach for HPV detection and genotyping in TSCC, and in particular on the methods that would best distinguish between clinically relevant HPV infections and “bystander” infections.

No pattern was noted in our review with regards to HPV prevalence in fresh-frozen vs. paraffin-embedded tissue, and all studies verified DNA integrity in their samples. Other authors have also concluded that the use of fresh-frozen or paraffin-embedded samples does not significantly affect HPV-positivity [31]. Small sample size could be responsible for some of the variability noted between studies. One must be cautious in comparing studies from different periods, as Näsmann et al. [3] have reported a rapidly increasing proportion of HPV-associated cases in Sweden even over a brief period (2003-2007). However, in France HPV prevalence did not change significantly between 2000 and 2009 [32]. Further data is needed to determine in which regions the proportion of HPV-positive cases is increasing, and at what rate, and the factors that are influencing this.

In the review by Kreimer et al. [31] on HPV prevalence in HNSCC worldwide, the reported incidence of oropharyngeal SCC was highest in Asia. The studies included were primarily from Japan. These
were not included in the present review, as data was not provided specifically for TSCC in these studies. In contrast, in our review the lowest HPV prevalence was in Asia (China (0%) and Taiwan (13%)). The low prevalence in the Chinese and Taiwanese studies is unlikely to be an artifact of the HPV-detection methods used, as these were well-established PCR-based methods. Furthermore, Li et al. [19] tested samples from both Australia and China with the same methodology and found a prevalence of 46% in Australia and 0% in China, suggesting a true difference in prevalence between the two locations. The number of samples tested from China was considerably smaller (as China has a very low incidence of TSCC), making results somewhat less reliable. Differences in the quality of the starting material cannot be ruled out, however are unlikely as a human gene marker was amplified for all samples, from both locations, to ensure adequate DNA quality. This regional contrast may be explained by true differences between the various Asian countries (i.e., genetic, environmental, behavioural).

No data based on HPV-detection in TSCC was available from Eastern Europe, South America, Africa or the Middle East. However, there is data to suggest that the prevalence of HPV in oropharyngeal cancer in these regions is low. Ribeiro et al. [17] have conducted a large multicenter study on patients with HNSCC (n=2214) and controls (n=3319) from regions with a high prevalence of HNSCC, such as Central Europe and Latin America. Seroprevalence to HPV E6, E7, and L1 was determined for various HPV types. Among tonsil carcinoma cases the seroprevalence of HPV16 E6 and E7 together was 8.9%, suggesting that HPV prevalence in TSCC is low. However patients with antibodies to both E6 and E7 had a > 30-fold increased risk compared to seronegative patients. Ribeiro et al. [17] also tested frozen tumor tissue from 136 oropharyngeal SCC cases from Latin America by PGMY09/11, and 68 of these were also tested using HPV 16 type-specific primers. The HPV DNA prevalence was low, with 0.7% (1/136) cases positive by PGMY09/11, and 4.4% (3/68) by HPV 16 type-specific PCR. All of the patients that had HPV-DNA in the tumor, also had E6 or E7 antibodies. Although E6 and E7 antibodies seem to be rather specific markers for oropharyngeal carcinoma, the sensitivity of these markers remains to be determined. It is significant to note that not all individuals exposed to HPV seroconvert or maintain detectable antibody levels over time.

Lastly, the data in tumor-free tonsillar tissue is limited, and insufficient to draw conclusions on regional differences, however it seems that the prevalence in the general population is low. Again heterogeneity in methodology makes it challenging to compare between studies. Of note, the age range of patients included varies considerably between studies. This is significant, as it seems to have an effect on HPV detection, with higher HPV prevalence detected in children/adolescents. Furthermore, some of the studies were small, and likely underpowered for detection of a low HPV prevalence.

Conclusions

The prevalence of HPV in TSCC is between 35-65% in the majority of studies from Western Europe, North America, and Australia, and considerably lower in China and Taiwan (0-13%). The incidence of TSCC in Western Europe, North America and Australia, has been increasing since the 1970’s. In Sweden and Norway this has been shown to be coincident with an increase in HPV-associated cases. TSCC-specific data is needed from Central/Latin America, Eastern Europe, as well as Africa and Asia. A multi-center study employing the same methodology across sites would be beneficial for providing a more accurate comparison across geographic sites. There are few studies on HPV prevalence in tumor-free tissue, however they indicate a prevalence of less than 10%. Further studies to better understand the epidemiology of tonsillar HPV infections and the natural history of these infections are needed.

As the predominant type in all studies is HPV16, it is likely that the currently available prophylactic HPV-vaccines will also be beneficial in the prevention of a certain proportion of TSCC cases. Likewise therapeutic vaccines currently in development for cervical and anogenital neoplasia could play a role in the future management of TSCC.

References

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