Oral squamous cell carcinoma patients which human papilloma virus infection: a case control study in Muwardi Hospital Surakarta, Central Java, Indonesia

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Abstract. Prayitno A, Aznar E, Poernomo, Putra STA. 2011. Oral squamous cell carcinoma patients which human papillomavirus infection: a case-control study in Muwardi Hospital Surakarta, Central Java, Indonesia. Nusantara Bioscience 3: 64-67. Annual incidence rates for oral and pharyngeal cancer are estimated at 25 cases per 100,000 in developing countries. Human papillomavirus (HPV) was implicated in pathogenesis of Oral Squamous Cell Carcinoma (OSCC). Aims of this research were to know the incidence of OSSC which realized HPV infection. Women OSCC (15) and Benign Oral Squamous Cells (BOSC) (40) tissue biopsy frozen sections were from Departement of Oral and Dental, Muwardi Hospital in Surakarta from January to December 2007. Tissue was cut into two parts. To ascertain the type of neoplasm was subsequently stains with HE. To amplify the L1 HPV gene for 450bp long. Chi-Square Test analyzed the collected data. The result of this experiment showed nine patients from 40 patients BOSC identified have HPV infections (9/40 = 23%). Eleven patient from 15 patient OSCC identified have HPV infections (11/15 = 73%). From Chi-Square analysis have significant differences between BOSC and OSCC. HPV is a factor for OSCC pathogenesis.

Keywords: developing countries, HPV, pathogenesis, OSCC, Moewardi Hospital.

INTRODUCTION

Factor that known implicated as a potential cock and or promoter cancer were tobacco, alcohol, radiation of sunrise, ionization radiation, carcinogen related work, environmental pollutant, medicines, nutrition, and infectious agent. Another factor is life in village, social-economic factor, age, gender and response immune mechaniam. Information about another factor was little. The followed factor is periodontal disease chronic, bed oral hygiene, diseases of tooth, sharp of set teeth, electrogalvanism and edentulism. Another researcher found that human papillomavirus (HPV), especially 16 and 18 types, implicated in oral squamous cell carcinoma (OSCC) pathogenesis (Bsoul et al. 2005). Risk Factors (account for 75% of cases) are tobacco abuse confers 6 fold risk, smokers represent 90% of oral cancer patients, alcohol abuse or heavy use, combined risk of heavy alcohol and tobacco use (women: 100 fold risk of oral cancer, men: 38 fold risk of oral cancer), other risks are sunlight exposure, poor dentition and viral infection (HSV, HPV) (Ravi and Yadav 2006; Ord et al. 2007).

The prevalence of oral cancer is also on the increase in Africa. Annual incidence rates for oral and pharyngeal cancer are estimated at 25 cases per 100,000 in developing countries. The rapid urbanization and increasing access to, and utilization of tobacco in its various forms as well as alcohol, is leading to an increase in the incidence of oral pre-cancer and cancer. Epidemiology of oral cancer is squamous cell represents 90% of oral cavity tumors, incidence increases with age and oral cancer is 9th most common cancer (represents 3% of cancers in men and represents 2% of cancers in women). The aims of this
research were to know the incidence of OSSC patient which realized HPV infection.

**MATERIALS AND METHODS**

Kind of this research is observational - , and the design of this research is posted test only control group design. Ethical clearance was done by dr Muwardi District Hospital Surakarta team and sign on August 5, 2008.

All patients were women. Forty biopsies froze section of Benign Oral Squamous Cells (BOSC) tissue patient and fifteen biopsies frozen section of Oral Squamous Cell Carcinoma (OSCC) tissue patients were collected from Oral and Dental Clinic of Muwardi Hospital in Surakarta, Central Java, Indonesia from January to December 2007.

Paraffin blocks were made from cutting I, which was subsequently stains with Haematoxyline Eosine (HE) to ascertain the type of neoplasm. Cutting II was subjected to DNA isolation. Deoxyribonucleic Acid (DNA) isolation was made by Schmits (1994) with some modifications. Cut up to 25 mg of tissue into small pieces, place in 1.5 mL microfuge tube volume, and add 200 ul of DNA extraction buffer. Add 20 µL of Proteinase K stock solution, mix by vortexing, and incubate at 55°C overnight.

The DNA isolation results were subjected to Polymerase Chains Reaction (PCR) to amplify L1-HPV for fixed the HPV. Diagnose related HPV infections are made by Schmits (1994) and McMillan and Fowler (1998). PCR method with some modifications (25 µL microfuge tube Ready To Go PCR Bead (Amersham Pharmacia Biotech) mixed with 2 µL HPV consensus primers (MY09: 5’GCAAAAACTATATGTATATGC3’ and MY11: 5’CTGCAAAAAGCTGGAATATC3’) (Cybergene AB) and 2 µL DNA template. PCR protocol for both amplifications are 94°C for 50 seconds, 59°C for 50 seconds, 72°C for 50 seconds and 4°C soak. The amplification very conserved region of HPV the L-1 gene that presents in all HPV subtypes produced 450 bp long.

The collected data was analyzed by Chi-Square Test (SPSS for Windows 15).

**RESULTS AND DISCUSSION**

The result of this experiment showed in Figure 1-3 and Table 1-2.

![Figure 1](image1.png)  
**Figure 1.** Histopathology view from HE stain of OSCC.

![Figure 2](image2.png)  
**Figure 2.** Polymerase Chains Reaction (PCR) L1-HPV gene in BOSC tissue frozen section, amplified 450 bp long.

![Figure 3](image3.png)  
**Figure 3.** Polymerase Chains Reaction (PCR) L1-HPV gene in OSCC tissue frozen section, amplified 450 bp long.

<table>
<thead>
<tr>
<th>Malignant</th>
<th>Age (years)</th>
<th>Benign</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Age (years)</td>
<td>No.</td>
<td>Age (years)</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>4</td>
<td>31</td>
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<tr>
<td>5</td>
<td>40</td>
<td>5</td>
<td>58</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>12</td>
<td>55</td>
</tr>
<tr>
<td>13</td>
<td>21</td>
<td>13</td>
<td>57</td>
</tr>
<tr>
<td>14</td>
<td>56</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>15</td>
<td>67</td>
</tr>
</tbody>
</table>

**Table 1.** The data women patient with OSCC (Malignant) and BOSC (Benign).

Mean = 50  
Mean = 42.6

<table>
<thead>
<tr>
<th>HPV positive</th>
<th>HPV negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSCC</td>
<td>11 (73%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>BOSC</td>
<td>9 (23%)</td>
<td>31 (77%)</td>
</tr>
</tbody>
</table>

**Table 2.** Result of the experiment in table 2X2

The result of this experiment showed mean for BOSC was 42.6years and OSCC was 50 years. And nine patient from 40 patient BOSC identified have HPV infections (9/40 = 23%). Eleven patient from 15 patient OSCC identified have HPV infections (11/15 = 73%).

Dental caries and periodontal disease are generally considered to be the major oral health problems around the world. In developing countries of Africa, these appear to be neither as common nor of the same order of severity as in

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Dental caries and periodontal disease are generally considered to be the major oral health problems around the world. In developing countries of Africa, these appear to be neither as common nor of the same order of severity as in...
the developed world. An epidemiological description of a
given health problem usually includes its prevalence,
severity (morbidity, mortality) and age-adjusted
distribution in the population. Oral diseases known to exist
in each community must be assessed in this way in order to
develop programmes appropriate to community needs.
Based on this form of analysis, the most prominent oral
health problems in Africa amongst low socio-economic
communities include Noma, ANUG (Acute Necrotising
Ulcerative Gingivitis), oral cancer, the oral manifestations of
HIV and AIDS, oro-facial trauma, and dental caries. The
highest global prevalence of HIV and AIDS is found in
Africa. Studies have shown that the oral manifestations of
HIV/AIDS are common. Candida infections, nongenital
gingivitis and oral hairy leukoplakia are the most common.
The prevalence of oral cancer is also on the increase in
Africa. Annual incidence rates for oral and pharyngeal
cancer are estimated at 25 cases per 100,000 in developing
countries. The rapid urbanization and increasing access to,
and utilization of tobacco in its various forms as well as
alcohol, is leading too (WHO/AFRO 2008).

In the case of high-risk HPV infection and under
favorable conditions, the viral genome is integrated into the
host genome which is the necessary event for the keratinocytes immortality. During this process of
integration the circular form of viral genome breaks at the
level of the E1 and E2 regions, never at the level of the E6
or E7 region. Different studies have shown that the
integrated part of the genome corresponds to E1, E6, and
E7 while the regions from E2 to E5 are lost and are not
transcribed in the tumors. The loss of E2 during this
process of integration produces the loss of E6 and E7
control. Therefore, the sequences E6 and E7 are directly
involved in the cellular cycle by inhibiting the normal
functions of p53 and pRb respectively. The protein p53 is
known as the "genome's guard," and in the case of DNA
damage, the p53 can provoke the arrest of cellular division
and assure the time necessary for DNA repair. If damage
can't be repaired, p53 is able to induce the programmed cell
death and prevent the propagation of DNA damage in
subsequent generations of cells.

In the case of other types of tumors, p53 is usually
mutated and acts as a real oncogene. In the case of HPV
infection, E6 suppresses the properties of p53 gene product
achieving the functional equivalent of the two hits required
to knock out both alleles of a tumor suppressor gene. The
mutations of p53 usually are not found. The E7 protein
interacts with retinoblastoma protein (pRb), which is the
crucial factor for the cell cycle control. This interaction
causes the release of the transcription factor E2F, which is
now free to act and can stimulate the cellular division. E7 is
also able to bind and inactivate the protein kinase inhibitors
p21 and p27 and can interact with different proteins whose
significance has still not been determined. E6 and E7 can
cooperate with cellular oncoproteins like ras and myc
which enables the virus to act at the level of growth factors
and cellular and nuclear metabolism producing oncogenic
cells. E6 and E7 can provoke DNA mutations of the host
cell directly, probably by causing alterations of DNA repair
mechanisms. This means that certain types of HPV are able
to cause malignant lesions even without the action of other
cofactors (González Intxaurraga et al. 2002).

Because X' (df=1; p<0.01) < X' (68.59) that showed in
Table 3, so that have significant differences between BOSC
and OSCC.

Table 3. Chi-Square data analysis of table 1.

<table>
<thead>
<tr>
<th>k.b</th>
<th>O</th>
<th>(O-E)</th>
<th>(O-E)^2</th>
<th>(O-E)^2/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1.r.1</td>
<td>11</td>
<td>14.5</td>
<td>-3.5</td>
<td>12.25</td>
</tr>
<tr>
<td>c.2.r.1</td>
<td>4</td>
<td>25.4</td>
<td>-20.6</td>
<td>424.36</td>
</tr>
<tr>
<td>c.1.r.2</td>
<td>9</td>
<td>5.4</td>
<td>3.6</td>
<td>12.96</td>
</tr>
<tr>
<td>c.2.r.2</td>
<td>31</td>
<td>9.5</td>
<td>21.5</td>
<td>462.25</td>
</tr>
<tr>
<td>X^2</td>
<td></td>
<td>68.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cancer is widely perceived as a heterogeneous group of
disorders with markedly different biological properties,
which are caused by a series of clonally selected genetic
changes in key tumor-suppressor genes and oncogenes.
However, recent data suggest that cancer has a
fundamentally common basis that is grounded in a
polyclonal epigenetic disruption of progenitor cells,
mediated by "tumor-progenitor genes". Furthermore, tumor
cell heterogeneity is due in part to epigenetic variation in
progenitor cells, and epigenetic plasticity together with
genetic lesions drives tumor progression. This crucial early
role for epigenetic alterations in cancer is in addition to
epigenetic alterations that can substitute for genetic
variation later in tumor progression. Therefore, non-
neoplastic but epigenetically disrupted progenitor cells
might be a crucial target for cancer risk assessment and
chemoprevention (Bsoul et al. 2005; Feinberg et
al. 2006).

The inducible transcription of heat shock genes is the
response to a plethora of stress signals, including (i)
environmental stresses, (ii) nonstress conditions, and (iii)
pathophysiology and disease states (e.g. HPV). Although
changes in heat shock protein (Hsp) expression are
associated with certain diseases, these observations leave
open the question of whether this is an adaptation to the
particular pathophysiological state, a reflection of the
suboptimal cellular environment associated with the
disease, or serves to warn other cells and tissues of
imminent danger (Morimoto 1998).

In the face of injury or stress with the use of various
mechanisms for anticipated, including systems of proteins
called molecular chaperones. The typical function of a
chaperone is to assist a nascent polypeptide chain to attain a
functional conformation as a new protein and then to assist
the protein's arrival at the site in the cell where the protein
carries out its functions. It has become increasingly clear
that disruption of chaperoning mechanisms contributes to
aging and disease. This review outlines the involvement of
defective chaperones in senescence and in several
diseases. Since chaperones are ubiquitous, their deficiencies
and defects are bound to affect diverse tissues and, hence, to be
of interest to those in internal medicine, ophthalmology,
neurology, immunology, endocrinology, pediatrics, and
gerontology. Only a fraction of chaperones is encoded in genes that are inducible by stressors and thus belong to the large class of stress proteins. OSCC was included in distress cell, so to make possible disturbane in protein folding process (Fan and Neff 2000; Rho et al. 2002; Martin 2004; Bonnet et al. 2007).

The accumulation of protein misfolded in distress condition to be the result of an increase of toxic functions, which are often accompanied by Hsp70 and other chaperones. No matter how toxicity is generated, either by soluble forms or insoluble fibrils of the disease proteins, the identification of protein aggregates, including Hsp70, inside or around dead cells has tempted many researchers to manipulate the level of Hsp70 to examine whether overexpression of chaperones would reduce the extent of aberrant aggregation, thereby suppressing disease phenotypes or delaying the onset of the diseases (Ellis 1996; Li et al. 2000; Muchowski et al. 2000; Soto 2003; Morishima 2005; Butler and Loh 2006; Park et al. 2007; Gruschus 2008; Gargari et al. 2009).

CONCLUSION

The result of this experiment showed that from BOSC patient identified to have 23% HPV infections and from OSCC patient identified to have 73% HPV infections. There have significant differences between BOSC and OSCC in HPV infection. The conclusion is HPV as a significant factor for OSCC pathogenesis.

ACKNOWLEDGMENTS

We thanks to acknowledging to Airlangga University in Surabaya for chance to study about cancer and so thanks to acknowledging to Sebelas Maret University for his laboratory facilities. We thanks AO Suryanata and Tri Darmani for their cooperation in collecting sample. We also thank Prof. Widya Asmara and Prof. Noerhayati Suripito for their inspiration.

REFERENCES


