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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 ST. 2011. Oral squamous cell carcinoma patients which human papilloma virus 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 papilloma virus (HPV) was implicated in pathogenesis of Oral Squamous Cell Carcinoma (OSCC). Aims of this research were to know the incidence of OSSC patient 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. The collected data was analyzed by Chi Square Test. 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.

Key words: developing countries, HPV, pathogenesis, OSCC, Moewardi Hospital.

Abstrak. *Prayitno A, Aznar E, Poernomo, Putra ST. 2011. Pasien oral squamous cell carcinoma dengan infeksi virus papiloma manusia: studi kasus kontrol di Rumah Sakit Muwardi Surakarta, Jawa Tengah, Indonesia. Nusantara Bioscience 3: 64-67.* Tingkat insiden tahunan untuk kanker mulut dan faring diperkirakan mencapai 25 kasus per 100.000 di negara-negara berkembang. Human papilloma virus (HPV) terlibat dalam patogenesis Oral Squamous Cell Carcinoma (OSCC). Tujuan penelitian ini adalah untuk mengetahui kejadian pasien OSSC yang mengalami infeksi HPV. Irisan beku biopsi jaringan OSCC (15) dan Benign Oral Squamous Cells (BOSC) (40) wanita diperoleh dari Bahian Mulut dan Gigi Rumah Sakit Muwardi Surakarta, dari bulan Januari sampai Desember 2007. Jaringan dipotong menjadi dua bagian. Untuk memastikan jenis neoplasma, maka diwarnai dengan HE. Untuk gen L1-HPV digunakan penanda berukuran 450bp. Data yang dikumpulkan dianalisis dengan Chi Square Test. Hasil penelitian ini menunjukkan sembilan pasien dari 40 pasien BOSC diidentifikasi mengalami infeksi HPV (9/40 = 23%). Sebelas pasien dari 15 pasien OSCC diidentifikasi mengalami infeksi HPV (11/15 = 73%). Dari analisis Chi Square terdapat perbedaan yang signifikan antara BOSC dan OSCC. HPV merupakan faktor patogenesis OSCC.

Kata kunci: negara berkembang, HPV, patogenesis, OSCC, Rumah sakit Muwardi.

INTRODUCTION

Factor that known implicated as a potential cock and or promoter cancer were tobacco, alcohol, radiation of sunrise, ionization radiation, carcinogen related work, environment pollutant, medicines, nutrition and infectious agent. Another factor is life in village, social-economic factor, age, gender and response immune mechanism. 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 papilloma virus (HPV), especially 16 and 18 type, 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 urbanisation 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 are 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 observasional - cross sectional and the design of this research is post test only control group design. Ethical clearance was done by dr Muwardi Distric Hospital Surakarta team and sign at August 5, 2008.

All patients was womens Fourty biopsy frozen section of Benign Oral Squamous Cells (BOSC) tissue patient and fiveteen biopsy frozen section of Oral Squamous Cell Carcinoma (OSCC) tissue patients collected from Oral and Dental Clinic of Muwardi Hospital in Surakarta, Central Java, Indonesia from January to December 2007.

Parrafin 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. Dioxyribonucleic 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 a 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 55^0 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'$GC_{(A/C)}CAGGG_{(A/T)}CATAA_{(C/T)}AATGC$3' and MY11: 5'$CGTCC_{(A/C)}A_{(A/G)(A/G)}GGA_{(A/T)}ACTATC$3') (Cybergene AB) and 2 µL DNA template. PCR protocol for both amplifications are 94^0C for 50 seconds, 59^0C for 50 seconds, 72^0C for 50 seconds and 4^0C soak. The amplification very conserved region of HPV the L-1 gene that present 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.

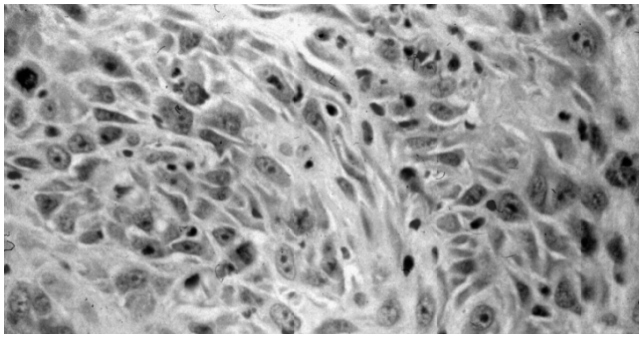

Figure 1. Histopathology view from HE stain of OSCC.

Figure 2. Polymerase Chains Reaction (PCR) L1-HPV gene in BOSC tissue frozen section, amplified 450 bp long.

Figure 3. Polymerase Chains Reaction (PCR) L1-HPV gene in OSCC tissue frozen section, amplified 450 bp long.

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

Malignant		Benign					
No.	Age (years)	No.	Age (years)	No.	Age (years)	No.	Age (years)
1	**39**	1	**30**	16	**15**	31	**47**
2	**53**	2	**40**	17	**54**	32	**45**
3	**69**	3	**45**	18	**40**	33	**49**
4	**60**	4	**31**	19	**27**	34	**51**
5	**40**	5	**58**	20	**27**	35	**51**
6	**45**	6	**20**	21	**19**	36	**53**
7	**42**	7	**19**	22	**28**	37	**43**
8	**73**	8	**27**	23	**67**	38	**44**
9	**50**	9	**27**	24	**31**	39	**41**
10	**45**	10	**40**	25	**45**	40	**70**
11	**43**	11	**15**	26	**32**		
12	**50**	12	**55**	27	**55**		
13	**21**	13	**57**	28	**65**		
14	**56**	14	**60**	29	**54**		
15	**70**	15	**67**	30	**61**		
Mean = 50				**Mean = 42.6**			

Table 2. Result of the experiment in table 2X2

	HPV positive	HPV negative	Total
OSCC	11 (73%)	4 (27%)	15
BOSC	9 (23%)	31 (77%)	40
	20	35	55

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

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, necrotizing 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 urbanisation and increasing access to, and utilisation 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 tumours. 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 cellular 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 are normally not found. The E7 protein interacts with retinoblastoma protein (pRb), which is the crucial factor for the cellular 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 directly DNA mutations of the host cell, 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^t (df=1; $p<0.01$) < X^2 (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.

k.b	O	E	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$
c.1.r.1	11	14.5	-3.5	12.25	0.84
c.2.r.1	4	25.4	-20.6	424.36	16.70
c.1.r.2	9	5.4	3.6	12.96	2.40
c.2.r.2	31	9.5	21.5	462.25	48.65
				X^2	68.59
				X^t (df = 1; p<0,01)	6.63

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 tumour-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 "tumour-progenitor genes". Furthermore, tumour cell heterogeneity is due in part to epigenetic variation in progenitor cells, and epigenetic plasticity together with genetic lesions drives tumour progression. This crucial early role for epigenetic alterations in cancer is in addition to epigenetic alterations that can substitute for genetic variation later in tumour 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 are 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 disturbance 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 over-expression 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; Garyali et al. 2009).

CONCLUSION

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

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