Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio: correlação com aspectos clínicos e fatores de risco

Human papillomavirus (HPV) detection in lip squamous cell carcinoma: correlation with clinical aspects and risk factors

Resumo

O papilomavírus humano (HPV) está associado a um largo espectro de lesões em humanos e tem sido ligado à carcinogênese oral. O objetivo deste estudo foi investigar a presença do DNA do HPV em pacientes com carcinoma espinocelular de lábio e correlacioná-la com aspectos clínicos e fatores de risco. Foram estudados 33 pacientes com carcinoma espinocelular de lábio. Destes, 30 pacientes foram positivos para o gene da beta-globina humana e então foram testados para o DNA do HPV com uso da reação em cadeia de polimerase em duas etapas (PCR e nPCR) com os oligonucleotídeos iniciadores MY11/MY09 e GP5+/ GP6+. O DNA do HPV foi detectado em 43,33% dos 30 pacientes analisados. Não houve associação com os fatores de risco analisados.

Palavras-chave: Epidemiologia. Investigação laboratorial. Carcinoma espinocelular. Papillomaviridae. Reação em cadeia da polimerase. Oligonucleotídeos.

Adriana Demathe[I]
José Fernando Garcia[II]
Neivio José Mattar[III]
Luciana Estevam Simonato[I]
Glauco Issamu Miyahara[I]

[I] Departamento de Patologia e Propedêutica da UNESP Araçatuba.

[II] Laboratório de Biologia molecular e Bioquimica do Curso de Veterinária da UNESP Araçatuba.

[III] Instituto de Patologia de Araçatuba – IPA.

Abstract

Human papillomavirus (HPV) is associated with a wide spectrum of lesions in humans, and it has been linked to oral carcinogenesis. The aim of this study was to investigate the presence of HPV DNA in patients with lip squamous cell carcinoma and to correlate it with clinical characteristics and risk factors. We studied 33 patients with lip squamous cell carcinomas. Of these, 30 were positive for human beta globin gene and tested for HPV DNA, using polymerase chain reaction in two steps (PCR and nPCR) with MY11/MY09 and GP5+/GP6+ primers. HPV DNA was detected in 43.33% of patients analyzed. There was no association with the risk factors analyzed.

Keywords: Epidemiology. Laboratory research. Squamous cell carcinoma. Papillomaviridae. Polimerase chain reaction. Oligonucleotides.

Introduction

According to the latest global estimate for 2008, cancer is responsible for approximately 7,6 million deaths per year[1]. Head and neck cancer is a collective term that defines malignant tumors located in upper aerodigestive tract. This anatomical region includes oral cavity, pharynx and larynx. About 40% of the head and neck carcinomas occur in the oral cavity (lip, base of tongue, tongue, gums, oral floor and palate)[2]. Squamous cell carcinoma (SCC) represents approximately 90% of all oral malignancies[3] and SCC of the lip comprising 25% to 30% of all oral sites[4].

Oral and oropharynx carcinomas accounting for 460,000 new cases and 250,900 deaths annually[5]. In Brazil, the estimated incidence of oral cancer for 2010, including lip, is 10.64/100,000 inhabitants/year among men and 3.76/100,000 inhabitants/year among women[6]. According to IARC[5] lip cancer incidence in Brazil for 2007 ranging of 0.8/100,000 (Brasília) to 1.4/100,000 (São Paulo) for man and ranging 0.1/100,000 (Cuiabá) to 0.5/100,000 (Goiânia) for woman.

The highest prevalence of lip SCC occurs between the sixth and seventh decades of life. The lower lip is the oral site more affected by SCC and usually diagnosed in stage I[7]. South of Australia is among the regions with the highest incidence of lip SCC, with 13.49 cases/100,000 inhabitants/year in men and 3.21 cases/100,000 inhabitants/year in women. In Europe, specifically in Spain, lip SCC represents a major impact on men (12.70 cases/100,000 inhabitants/year), and in Switzerland presents the highest incidence in women (0.83 cases/100,000 inhabitants/year)[8]. In France, the lip, mouth and pharynx cancer incidence reaches 38.5/100,000[9]. In Brazil, according to the estimate of National Institute of Cancer for 2008, the incidence of oral cancer was 14.45/100,000 in men and 4.83/100,000 in women[6].

Oral cancer etiology is multifatorial and the main risk factors are smoking and alcohol consumption[10]. Moreover, about 10% to 20% of patients with oral cancer are

Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.
99
Rev Bras Epidemiol
2011; 14(1): 98-105

non-smokers and non-drinkers suggesting that other factors, including certain viruses, may have implications on the oral carcinogenesis[11,12]. Among the viruses that appear to be related to oral cancer etiology is the human papillomavirus (HPV)[11-13]. HPV role in the etiology of cervix carcinoma is clear, however its participation on carcinogenesis in oral cavity and other anatomical sites such skin, esophagus, paranasal conjunctiva, bronchus, larynx, oropharynx only is suggested[13]. Syrjänen and cols. described in 1983[14] a possible link between HPV and oral cancer due to the observation of cytopathic alterations in oral lesions similar to those typically induced by HPV previously found in cervix cancer. Regarding the significance of HPV DNA presence in prognosis of patients with oral SCC, literature data are controversial[15-18]. However, its has been suggested differences between patients infected and no infected with HPV in relation to sex, age, tumor regions, histological grade, regional metastasis, rate of recurrence and survival[18]. Due to the great controversy in the literature, further studies are needed to help clarify the remaining questions that still persist. The aim of this study was to investigate HPV DNA presence in biopsies of lip SCC and correlate with clinical aspects and risk factors.

Materials and Methods

This study was reviewed and approved by Research Ethics Committee of São Paulo State University – Dentistry School of Araçatuba. Thirty-three caucasian patients presenting lip SCC confirmed by histological diagnosis participated in the study.

A questionnaire was completed at the initial patient trial asking about age, sex, occupation, tobacco and alcohol consumption and sun exposure. If the patient had oral cancer and none of this risk factors above the risk factor is described as "other risk factors". Smokers were considered those that smoked regularly during any time of life, even those who stopped. Non-smokers were those who never used tobacco. The same criteria were used for drinkers and non-drinkers classification. Information about TNM classification, location site of the primary tumour and degree of histological differentiation were obtained. The TNM classification of malignant tumours is a cancer staging system that describes the extent of cancer in a patient's body: "T" describes the size of the tumor and whether it has invaded nearby tissue, "N" describes regional lymphnodes that are involved and "M" describes distant metastasis.

All biopsy specimens were graded by the same pathologist according to Broders[19].

DNA extraction

Sections were obtained from 33 paraffin blocks in order to obtain 25 mg of material. DNA extraction was performed with QIAamp DNA Mini Kit® (QIAGEN Ltd, Crawley, UK) according to manufacture's instructions. Purity of DNA was accessed spectrophotometrically (NanoDrop® ND-1000 UV-Vis) and samples stored at -20°C.

PCR amplification

From thirty three cases, three did not show positivity for beta globin gene after PCR using primers GH20 and PC04 (Invitrogen Life Technologies®, Brazil) as reported by Bell et al.[20]. Therefore thirty cases were employed in this study. After confirmation of the presence and integrity of genomic human DNA, 150 to 300 ng of DNA were tested for HPV DNA by PCR. In first PCR round a mixture with 0,02 mM of each degenerate consensus primers[21]. MY11 and MY09 (Invitrogen Life Technologies®, Brazil), was used to amplify fragments of 450 bp from L1 late region of viral genome. The remaining reaction components were: 10.9 microlitres of ultra-pure water, 2.5 microlitres PCR buffer 10X (Invitrogen Life Technologies®, Carlsbad, CA, EUA), 4mM MgCl2 (Invitrogen Life Technologies®, Carlsbad, CA, EUA), 15 pmol dNTPs and 1 unit of Platinum Taq DNA polymerase (Invitrogen Life Technologies®, Carlsbad, CA, EUA). Approximately 150-300

Rev Bras Epidemiol
2011; 14(1): 98-105 100 Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.

ng of genomic DNA from each sample were added to mixture. Same quantity of Hela cells, with up to 4 copies of HPV-18 per cell, was used as positive control for HPV infection. The negative control was composed by all PCR components except DNA. The mixture was allowed to 94ºC for 10 min, 40 PCR cycles (94ºC for 1 min; 55ºC for 1 min; 72ºC for 40) and 72°C for 4 minutes. Two microlitres of the product from the PCR reaction were used in all thirty samples for nested PCR (nPCR) reaction directly in a microtube containing: 0,02 mM of each primer GP5+ and GP6+(Invitrogen Life Technologies®, Brazil), which produce a 150 pb DNA fragment[22]. The remaining reaction components and conditions were as described for the first round of PCR, except the annealing temperature that was reduced to 43ºC. Ten microlitres of the nPCR products were fractionated by electrophoresis in a 8% polyacrylamide gel, for 3 hours at 100 volts. Band visualization was performed by staining with silver nitrate solution. Samples were scored as either HPV DNA-positive or negative based on inspection of silver nitrate stained bands. PCR amplification was performed in triplicates for each sample. Samples were classified in positive or negative based on gel analysis. Presence or absence of HPV DNA were correlated with the following variables: gender, age, clinical staging, histologic grade (cell differentiation), survival, consumption of alcohol, smoking and solar radiation exposition.

Statistical analysis

The data were analyzed using Qui-square test and Fisher's exact test with Epi Info 6 Statistical Analysis System Software (1997, Centers for Disease Control and Prevention, USA). Differences were considered to be statistically significant when *P* values were less than 0.05. Survival rate was measured in years, comprehending the period from diagnosis date until death, confirmed by death certificate, or the last contact for alive patients. Kaplan Meyer was employed for global and free disease survival.

Results

In this study thirthy three patients were selected, but three were not positive for β-globin (without DNA), then thirty samples have been analyzed by PCR and re-analyzed by nPCR. Overall, HPV DNA sequences were not amplified by PCR, but were amplified by nPCR in 43.33% of the samples (Figure 1). There was not statistical significance between demographic characteristics of patients and HPV DNA presence (Table 1). Disease-free survival average was 9.3 years. The survival rate of 5 years was 89.6% and showed 9.54 and 9.14 years for HPV DNA positive and negative groups, respectively (Figure 2). There was only one death by lip SCC. There was not statistical difference between mean survival rate of HPV DNA positive and negative groups.

Discussion

Head and neck SCC are correlated with broadly varying rates of HPV DNA incidence worldwide[23]. These differences in the detection of HPV DNA in oral SCC suggest a potential difference of PCR based methodology in the ability to amplify fragments of different sizes and specific types of HPV, according to the DNA detection method used. They can also be the result of different material sources (smears, fresh, frozen, paraffin embedded), anatomic site, diverse population, PCR conditions, design of primers and number of samples analyzed. PCR method used in this study is one of the most sensitive to detect HPV DNA[3].

The primers generally used in HPV DNA researches are MY09/MY11[21], GP5/GP6[24] or GP5+ / GP6+[22]. PCR with consensus primers M09/MY11 has shown to be less sensitive compared to the PCR in two stages (nPCR)[25-27], employed in the present study.

PCR studies with paraffin-embedded tissues have showed worst results when compared with nPCR[28-30], fact ratified in this study.

Differences between populations with oral SCC were reported in a systematic review

Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.

101

Rev Bras Epidemiol
2011; 14(1): 98-105

Tabela 1 – Relação entre a presença do DNA do HPV, variáveis clínico-patológicas e fatores de risco em pacientes com carcinoma espinocelular de lábio.
Table 1 – Relationship between presence of HPV DNA, clinical-pathological variables and risk factors for patients with lip squamous carcinoma.

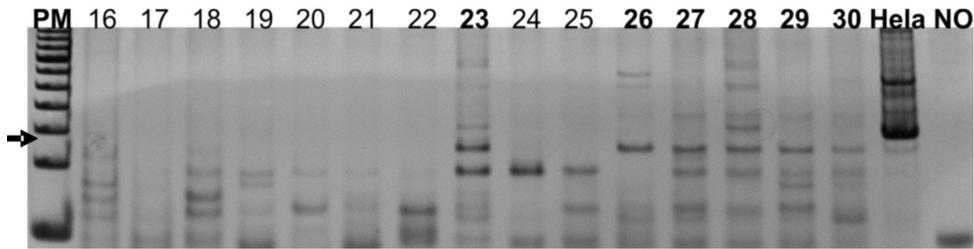| Variáveis	Pacientes (%) (n=30)	HPV + (%) (n=13)	HPV - (%) (n=17)	P*
Gênero				
Masculino	26(86,7)	10(76,9)	16(94,1)	
Feminino	04(13,3)	03(23,1)	01 (5,9)	0,20
Idade				
<60	14(46,7)	06(46,2)	08(47,1)	
≥60	16(53,3)	07(53,8)	09(52,9)	0,96
Estadiamento				
I	18(60,0)	07(53,8)	11(64,7)	
II	05(16,7)	04(30,8)	01 (5,9)	0,27
III/IV	07(23,3)	02(15,4)	05(29,4)	
Grau histológico				
Bem diferenciado	09(30,0)	05(38,5)	04(23,5)	
Moderadamente diferenciado	20(66,7)	08(61,5)	12(70,6)	0,49
Pouco diferenciado	01(3,3)	--	01 (5,9)	
Exposição solar				
Sim	23(76,7)	08(61,5)	15(88,2)	0,10
Não	07(23,3)	05(38,5)	02(11,8)	
Tabagismo				
Sim	19(63,3)	07(61,5)	12(64,7)	
Não	11(36,7)	04(38,5)	07(35,3)	0,86
Etilismo				
Sim	15(50,0)	09(69,2)	06(35,3)	
Não	15(50,0)	04(30,8)	11(64,7)	0,06
Outros fatores de risco	02(6,67)	02(100)	0(0)	

χ² ou Teste de Fisher

of studies that evaluated HPV DNA detection methods used in several continents[23]. For European and Asian countries the rates ranged between 16% and 33%[23], but when considered the Latin American population the rates ranged between 50% and 60% for oral SCC[31,32]. We agree with Cañadas[33] that reports that infection with HPV DNA is directly linked to early sexual experience, number of sexual partners and sexual contact with multiple partners, referring that these facts can increase the rates of HPV infection.

We believe that lip SCC is an oral pathology that has it own specific behavior, therefore has a particular HPV infection rate.

Few studies evaluated exclusively lip SCC, including this location among all oral SCC, were most of them ranged from 1 to 7 cases with HPV DNA detection range from 0% to 33.34%[17,28,35,36]. The largest studies evaluating HPV DNA detection of in lip SCC was a multicenter study held in Sweden, Norway and Finland, which included 57 cases and found 4% DNA HPV positive, in a population greatly different from of latin american[35].

Detection rate of HPV DNA similar to present work was found by Riethdorf[15] that evaluated 18 cases of lip SCC by PCR using frozen tissues and found 50% of positivity.

Regarding the survival rate of 5 years in

Rev Bras Epidemiol
2011; 14(1): 98-105   102   Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.

PM= peso molecular (50 pb); HeLa (controle positivo para DNA do HPV) e NO (controle negativo). A seta indica a posição aproximada esperada das amostras positivas.
PM= molecular weight (50 pb); HeLa (positive control for HPV DNA) and NO (negative control). The arrow shows the expected approximate position of positive samples.

Figura 1 – *Representação da eletroforese em gel de poliacrilamida 8% mostrando a amplificação do DNA do HPV pela nested PCR com DNA de amostras de carcinoma espinocelular de lábio.*
Figure 1 – *Representation of 8% polyacrylamide gel electrophoresis, showing the amplification of HPV DNA by nested PCR with DNA of lip squamous cell carcinoma.*
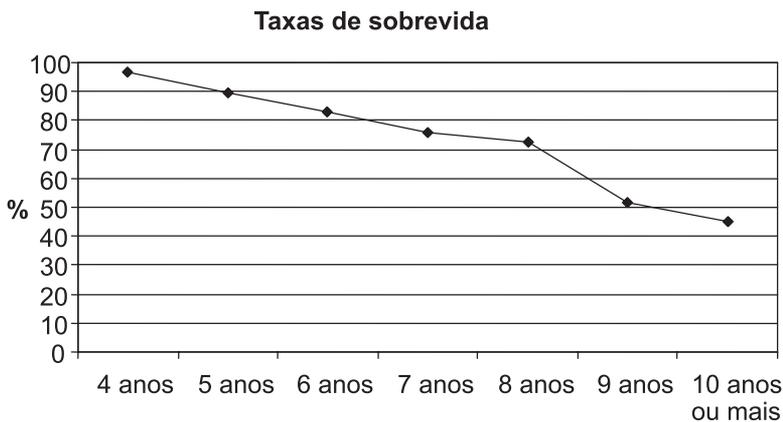

Figura 2 – *Taxas de sobrevida para câncer de lábio.*
Figure 2 –*Survival rates for lip cancer.*

lip SCC, Ogura[16] presented 82.5%, despite the fact that his study had 75% of the lesions with clinical staging I and about 43% were well differentiated. In our study 60% of the lesions were in stage I and 30% were well differentiated, despite that survival rate was higher (89.6%).

As for the other variables (gender, age, clinical staging, histologic grade, tobacco and alcohol) there was no statistically significant difference in the association of any of these variables and the presence of HPV. This lack of association between HPV infections and these variables was also observed in other studies[15,36]. We believe that the lip can be only a reservoir for the human papillomavirus.

The association nearest to be statistically significant was between the presence of HPV in patients alcoholism (p= 0.06544). It is possible that with a higher patient number we could find a significant correlation.

An interesting finding in this study was that two HPV positive cases had no exposure to any of the etiological factors evaluated (solar radiation, use of tobacco, and alcohol), justifying further investigation to elucidate the role of HPV.

Our findings showed that the presence of HPV DNA was detected in 43.33% of samples of lip SCC samples studied. The presence of viral particles of HPV DNA had no correlation with age, sex, smoking and consumption of alcohol, solar radiation, clinical staging, histological grade or survival, nevertheless more studies are necessary to understand the real HPV role in lip carcinogenesis.

Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.
103
Rev Bras Epidemiol
2011; 14(1): 98-105

References

1. Boyle P, Levin B. International Agency of Research Against Cancer. *World cancer report 2008.* Boyle P, Levin B, eds. Lyon: IARC Press; 2008.

2. Döbrössy L. Epidemiology of head and neck cancer: magnitude of the problem. *Cancer Metastasis Rev* 2005; 24: 9-17.

3. Luo CW, Roan CH, Liu CJ. Human papillomaviruses in oral squamous cell carcinoma and pre-cancerous lesions detected by PCR-based gene-chip array. *Int J Oral Maxillofac Surg* 2007; 36 153-8.

4. Hasson O.Squamous cell carcinoma of the lower lip. *Oral Maxillofac Surg* 2008; 66(6): 1259-62.

5. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M et al. *Cancer Incidence in Five Continents*, Vol. IX. IARC Scientific Publications No. 160, Lyon: IARC; 2007.

6. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância. Estimativas 2010. *Incidência de câncer no Brasil.* Rio de Janeiro: INCA; 2010.

7. Visscher JGAM, Schaapveld M, Otter R, Visser O, van der Wall I. Epidemiology of cancer of the lip in the Netherlands. *Oral Oncol* 1998; 34: 421-6.

8. Fernández-Ángel I, Rodríguez-Archilla A, Aneiros Cachaza J, Muñoz Medina M, Serrano Ortega S. Markers of metastasis in lip cancer. *Eur J Dermatol* 2003; 13: 276–9.

9. Ménégoz F, Lesec'h MJ, Rame JP, et al. Les cancers de la lèvre, de la cavité buccale et du pharynx en France: incidence, mortalité et tendance (période 1975-1995). *Bull Cancer* 2002; 89: 419-29.

10. Herrero R, Castellsagué X, Pawlita M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *J Natl Cancer Inst* 2003; 95: 1772-83.

11. Gillison ML, Koch WM, Capone RB, et al. Evidence for a casual association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000; 92: 709-20.

12. Gillison ML, Shah KV. Human papillomavirus-associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers. *Curr Opin Oncol* 2001;13: 183-8.

13. Syrjänen S. Human papillomaviruses in head and neck carcinomas. *N Engl J Med* 2007; 356: 1993-5.

14. Syrjänen K, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 1983; 12: 418-24.

15. Riethdorf S, Friedrich RE, Ostwald C, et al. p53 gene mutations and HPV infection in primary head and neck squamous cell carcinomas do not correlate with overall survival: a long term follow-up study. *J Oral Pathol Med* 1997; 26: 315-21.

16. Ogura I, Amagasa T, Iwaki H, Kijima T, Kurabayashi T, Yoshimasu H. Clinicopathological study of carcinomas of the lip and the mucosa of the upper and lower lips. *Int J Clin Oncol* 2001; 6: 123-7.

17. Paz IB, Cook N, Odom-Maryon T, Xie Y, Wilczynski SP. Human papillomavirus (HPV) in head and neck cancer: an association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. *Cancer* 1997; 79: 595-604.

18. Alvarez Alvarez I, Sanchez Lazo P, Ramos Gonzalez S, Rodrigo Tapia JP, Nunez Batalla F, Suarez Nieto C. Using polymerase chain reaction to human papillomavirus in oral and pharyngolaryngeal carcinomas. *Am J Otolaryngol* 1997;18: 375-81.

19. Broders AC. Carcinoma: grading and pratical applications. *Arch Pathol Lab Med* 1926; 2: 376-81.

20. Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (GSTM1) that increases susceptibility to bladder cancer. *J Natl Cancer Inst* 1993; 85: 1159-64.

21. Manos MM, Ting Y, Wright DK, Lewis AI, Broker TR, Wolinsky SM. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989; 7: 209–14.

22. De Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papilomavírus detection by PCR. *Gen Virol* 1995; 76: 1057-62.

23. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papilomavírus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 467–75.

24. Uobe K, Masuno K, Fang YR, et al. Detection of HPV en Japanese and Chinese oral carcinomas by in situ PCR. *Oral Oncol* 2001; 37: 146-52.

25. Evander M, Edlund K, Bodén E, et al. Comparison of a one-step and a two-step polymerase chain reaction with degenerate general primers in a population based study of human papillomavirus infection in young Swedish women. *J Clin Microbiol* 1992; 30: 987-92.

26. Husnjak K, Grce M, Magdic L, Pavelic K. Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. *J Virol Methods* 2000; 88: 125-34.

27. Remmerbach TW, Brinckmann UG, Hemprich A, Chekol M, Kuhndel K, Liebert UG. PCR detection of human papillomavirus of the mucosa: comparison between MY09/11 and GP5+/6+ primer sets. *J Clin Virol* 2004; 30: 302-8.

28. Bouda M, Gorgoulis VG, Kastrinakis NG, et al. "High risk" HPV types are frequently detected in potentially malignant and malignant oral lesions, but not in normal oral mucosa. *Mod Pathol* 2000; 13: 644-53.

29. Chang JY, Lin MC, Chiang CP. High-risk human papillomaviruses may have an important role in non-oral habits-associated oral squamous cell carcinomas in Taiwan. *Am J Clin Pathol* 2003; 120: 909–16.

30. Brestovac B, Harnett GB, Smith DW, Frost F, Shellam GR. Multiplex nested PCR (MNP) assay for the detection of 15 high risk genotypes of human papillomavirus. *J Clin Virol* 2005; 33: 116-22.

31. Premoli-De-Percoco G, Ramirez JL. High risk human papillomavirus in oral squamous carcinoma: evidence of risk factors in a Venezuelan rural population. Preliminary report. *J Oral Pathol Med* 2001; 30: 355-61.

32. Correnti M, Rivera H, Cavazza ME. Detection of human papillomaviruses of high oncogenic potential in oral squamous cell carcinoma in a Venezuelan population. *Oral Dis* 2004; 10: 163-166.

33. Cañadas MP, Bosch FX, Junquera ML, et al. Concordance of Prevalence of Human Papillomavirus DNA in Anogenital and Oral Infections in a High-Risk Population. *J Clin Microbiol* 2004; 42: 1330-2.

34. Elamin F, Steingrimsdottir H, Wanakulasuriya S, Johnson N, Tavassoli M. Prevalence of human papillomavirus infection in premalignant and malignant lesions of the oral cavity in U.K. subjects: a novel method of detection. *Oral Oncol* 1998; 34: 191-7.

35. Smith EM, Ritchie JM, Summersgill KF, et al. Age sexual behavior and human papilomavírus infection in oral cavity and oropharyngeal cancers. *Int J Cancer* 2004; 108: 766-72.

36. Cruz IB, Snijders PJ, Steenbergen RD, et al. Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas. *Eur J Cancer B Oral Oncol* 1996; 32B: 55-62.

Detecção do papilomavírus humano (HPV) em carcinoma espinocelular de lábio
Demathe, A. et al.
105
Rev Bras Epidemiol
2011; 14(1): 98-105