Case Report

Contact Endoscopy (Stomatoscopy) Versus Histological Diagnosis of Lesions of the Lower Lip Vermilion Area

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Abstract

In Brazil, the incidence of oral cavity cancer is estimated at 14,160 new cases in 2008. Contact endoscopy (stomatoscopy) applied to the oral cavity may favor early diagnosis. The aim of this study is to compare the contact endoscopy diagnosis to histological diagnosis of lesions of the lower lip vermilion area. Fifty-three prospective, *ex vivo*, non-consecutive lesions of the lip vermilion area were stained with toluidine blue solution, examined with stomatoscopy and directly biopsied. False-negatives did not occur and false-positives represented 9.4%. Sensitivity to diagnosis of malignancy was 100%, specificity was 88%, positive predicted value was 70.5%, negative predictive value was 100% and accuracy was 90.3%. Sensitivity and specificity of the contact endoscopy were high and the positive predictive value in relation to diagnosis of malignant lesions was good. Thus, stomatoscopy may be useful to diagnose lesions of the lower lip vermilion area.

Keywords: Mouth neoplasms, Oral neoplasms, Lip neoplasms, Endoscopy/methods, Early diagnosis

Introduction

Oral cavity cancer, which affects mainly men, is among the ten most common forms of cancer in the world.[1] In the United States, the expectation of new cases and deaths caused by oral cancer in 2007 was estimated at 22,560 and 5,370, respectively.[2] In Brazil, the incidence of oral cavity cancer is estimated at 14,160.[3] Approximately 50% of such tumors are diagnosed belatedly (stage III or IV).[4]

Cytodiagnosis may favor diagnosis and toluidine blue may orient biopsy of questionable lesions on clinical examination;[5-6] however, such methods are not totally safe because of their false-positives or false-negatives rates. Therefore, new reliable non-invasive procedures are necessary in order to obtain early diagnosis of malignant lesions, *in vivo*.[7]

Colposcopy with optical enlargement of 40 times was applied in order to favor the diagnosis of oral cavity lesions,[8] with satisfactory results, mainly when associated with toluidine blue test, which is used in stomatoscopy.[9-10] Another instrument used by gynecology and adapted for oral cavity use is microcolpohysteroscopy, patented by Hamou in 1979 for examinations of the epithelium of cervix uteri and with enlargements of 60 and 150 times.[11-12]

This method was applied to other

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topographies,[13-18] including oral cavity mucosa. L'Estrange, in 1989,[19] mentioned that such a method might be the bridge between microscopy of the mucosal surface and histology. In addition, he emphasized that other studies would be necessary in order to establish the level of the existing correlation between contact endoscopy and histology. From 2004 on, other studies related to the oral cavity were presented to the medical community concerning benign[20] and/or malignant [21] lesions.

All studies are based on previous known alterations by cytopathology, such as nuclear alterations and the consequent increase of nucleus-cytoplasm relation, high cell density per field, atypical or normal mitosis on the superficial layer, changes of the distribution pattern of the superficial layer of epithelium, the loss of cell maturation in this layer, etc.[22-26] Regardless of the diagnostic method used, a positive result shall be confirmed by biopsy. According to a previous study, the criteria used for the analysis of alterations found through stomatoscopy of lesions of the cervix uteri, larynx and rhinopharynx, for instance, are applied to oral and lip lesions.

The aim of this study is to compare the stomatoscopy diagnosis of the lesions of the lower lip vermilion area to the histological diagnoses (gold standard), and to calculate the sensibility, the specificity, the positive and negative predictive values and the accuracy of the exam.

Methods

The project was approved by the Hospital Ethics and Research Committee of Hospital Erasto Gaertner (approval date: 5 April 2005; project id: pp-1149).

This was a prospective non-consecutive case study of lower lip vermilion area lesions; furthermore, it was accomplished between December 2005 and July 2007, only *ex vivo* specimens were analyzed, *i.e.*, from surgical specimens which were product of resections of lower lip vermilion area malignant lesions (squamous cell carcinoma), which were not preserved in aqueous solution formaldehyde, in the operating room Sixteen patients with squamous cell carcinoma (16 surgical specimens) from the Outpatient Clinic of the Head and Neck Surgery Service ,were included in this study. No patient had been submitted to locoregional radiotherapy, previous chemotherapy or surgery. All patients received information about the study routines and written informed consent was obtained.

There were a total of 58 contact stomatoscopy examinations, more than three per each surgical specimen. Five of them did not have a concluded histological diagnosis (a poor-quality or insufficient sample) and/or did not have the corresponding images (recording) in a position to be evaluated, and were subsequently excluded. Therefore, 53 examinations were used in the assessment in relation to benignity and malignancy, in the same location where the biopsy was done. The entire process, including biopsies and analyses, was performed by the same physician, a Head and Neck surgeon, with pathologists as advisors.

Equipment

The device used in this study was a Karl Storz contact laryngoscope 8715 A, angulation of 0°, diameter of 4mm, providing enlargements of 60 and 150 times. The camera used was a Karl Storz 202321 – 20, the light source was a Karl Storz Xenon 175, the VCR was a Sony SLV 40 BR, the image digitalization was accomplished by Sansung DVD Recorder DVDR 150, the TV set was a Sony KV 1450 B, and the computer used was Toshiba A 55 Series.

Technique applied

The examination consisted of the endoscope lens in contact with the mucosa, the vermilion area and/or with the lesion. The staining routine consisted of: local hygiene with a moist gauze saline solution, applied with moist gauze; one-minute application of 1% acetic acid; one minute application of 1% toluidine blue, and; removal of the excess staining with an embedded gauze saline solution.

Area of image and biopsy

Stomatoscopy -guided biopsies were taken directly from the lesion from areas located at 12, 3 and 9 o'clock of the perimeter of each lesion (4 mm from the tumor and normal mucosa transition). Thus, the findings and the diagnosis obtained by the images of both the previously diagnosed tumor and the surrounding mucosa (without signs of malignancy) were compared with the corresponding histological diagnosis.

Criteria for imaging analysis

Imaging analysis used the criteria of quality, histological aspects (cellularity, architecture and stratification) and cytological aspects.

Quality was classified as sufficient or non-sufficient. Sufficient was defined when it was possible to visualize or at least identify the surface feature of the lower lip vermilion area, which usually does not have nuclei, and from there the changes identified by the method under magnification of 60 and 150 times.

Histological aspects included: cellularity, classified as normal or increased, based on the number of cells of the normal lip mucosa or lip vermillion area, of the same patient, under magnification of 150 times, compared with the number of cells of the lesion examined [27] (the authors considered that the presence of more than 50 cells indicated malignancy); architecture, classified as normal or altered. It is the cell distribution, *i.e.*, the superficial cell arrangement under magnification of 60 and 150 times (the regular distribution of cells was considered normal), and; stratification, classified as normal when the superficial layer of the lip vermilion area had mature cells (with little or no nuclei and abundant cytoplasm). Therefore, it was considered abnormal when we found cells of the basal and/or intermediate layer of mucosa. The classification was made according to the observation of the examiner.

Cytological aspects, or morphometry, were considered abnormal if there were altered nucleus shape, evident staining concentration, increase of the nucleus-cytoplasm proportion, or the presence of hyperchromatic nucleus and mitotic figures. Hence, morphometry was classified as normal if such characteristics had not been collectively or individually found.[27]

The combination of criteria considered for the diagnosis of malignancy was increased cellularity and amended stratification, architecture and morphometry.

Statistical Analysis

Variables were analyzed using descriptive statistics, regression analysis and Kendall's tau (τ) coefficient through the software Statistical Package for Social Sciences (SPSS), version 12.0. The level of signification adopted for the statistical tests was 5% ($p < 0.05$).

Assessment of Stomatoscopy Performance

Stomatoscopy diagnoses were compared to the anatomopathological diagnoses (gold standard). Additionally, sensitivity, specificity, positive and negative predictive values, and accuracy were calculated

Results

Among the 53 examinations performed, 77.4% (12) had histological diagnosis of benignity and 22.6% (41) of squamous cell carcinoma. Kendall's correlation test regarding all criteria was $p < 0.05$.

Comparing the stomatoscopy diagnoses of lower lip vermilion area lesions to the histological diagnoses, as shown in Table 1, 12 true-positives, 5 false-positives, no false-negative and 36 true-negatives were obtained. The sensitivity was 100%, the specificity was 88%, the positive predicted value was 70.5%, the negative predictive value was 100%, and the accuracy was 90.3 %.

Table 1 - Final Study: Distribution of each two, of diagnosis of contact microstomatoscopy and anatomopathological diagnosis of lower lip vermilion area, not including those 16 cases of mucosa with no lesion.

	AP +	AP -	TOTAL
EXAMINATION +	12(22.6 %)	5(9.4 %)	17(32 %)
EXAMINATION -	0	36(67.9 %)	36(68 %)
TOTAL	12(22.6 %)	41(77.4 %)	53(100 %)

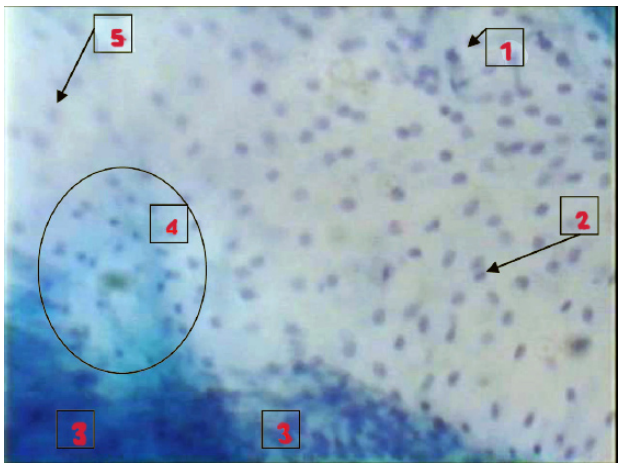
AP = Anatomopathological diagnosis; + = positive; - = negative

Discussion

The criteria used in this study are already enshrined by cytopathologists [28] and histopathologists[26], besides being used and mentioned by previous studies of contact endoscopy.[10,18,23-25]

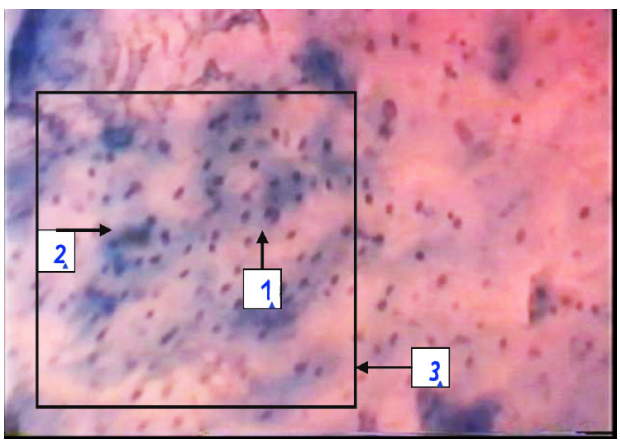
These criteria are likely to be identified in both *in vivo* and *ex vivo* cases because they do not depend on natural color, but the color provided by the toluidine blue staining (Figures 3 and 4).[29] Moreover, the vascular changes of the lesion and its surroundings were not considered in this study, which in principle would be more visible in *in vivo* cases.

Ex vivo cases were chosen since it facilitates the biopsies, both as to the number and direction. As these surgical specimens were of patients who had a diagnosis of squamous cell carcinoma, biopsies were directed to the malignant lesion and the regions around (4 mm away). The intention was to detect small changes (cellularity,



Legend -The main criteria and structures (except vessels) to be analyzed are evident: 1 – cell in metaphase; 2 - cells in cytokinesis, 3 - tumor area, probably with keratin, difficult to discern the cytoplasm and / or nucleous. Increased cellularity; 4 – nuclei with smaller area, may be atypical, 5 - blurring probably by stretching the tissue at the time of contact with the device. Source: Ramos et al. (2006)52.

Figure 1 – Microstomatoscopy, ex vivo,150 x: mitosis, increased cellularity, nuclear changes



Legend- The main structures analyzed in figure 1, ex vivo, may also be highlighted, now in vivo: 1 – cells in cytokinesis; 2 – tumor area, difficult to discern the cytoplasm and / or nucleous; 3- Increased cellularity. Smaller Nuclei, may be atypical.Source: Researcher

Figure 2 - Ulcerated lesion of the vermilion of the lower lip (squamous cell carcinoma), in vivo, 150 x

architecture and morphology) that occur in the mucosa around the lesion because it may represent an early stage or microscopic manifestation of carcinogenesis, which can be used to assess patients with minor but suspicious *in vivo* lesions.

In 1989, P. L'Estrange affirmed that microcolpohysteroscopy could be a reliable approach to detailed observation of the surface of the oral tissues. However, he did not compare the contact endoscopy diagnoses of oral lesions with the histological diagnoses. Pak, in 2002,22 evaluated the potential use of contact endoscopy for the diagnosis of persistent and recurrent nasopharyngeal carcinoma after radiotherapy and found 92.1% diagnostic accuracy. This result is similar to the results of this study concerning the lip vermillion area (90.3%).

Other tests, such as cytodiagnosis and toluidine blue, were studied through different methodologies and diverse calculi of assessment.[30] The sensitivity of the toluidine blue test ranged from 78 to 100% and the specificity from 31 to 100%.[31] Cytodiagnosis presented low sensitivity with large number of false-negatives,[30] obtaining only 23% in a Brazilian study.[9] Despite this, they are used in the suspicion, detection and diagnosis of malignant lesions of the oral cavity.The sensitivity of the stomatoscopy was 100%, therefore, the test has a great capacity to detect malignant lesions of the lip vermillion area. Nonetheless, it may also lead to false-positives, requiring an association of the clinical characteristics of the lesion and medical monitoring. False-negatives did not occur, showing that the method is very useful to exclude malignancy when it is faced with a benign lesion (100% of negative predictive value).

According to Lingen et al.[31] a screening test must have the five characteristics of being: simple, safe and accepted by the population; able to detect the disease early in its natural evolution; able to preferentially detect those lesions that are prone to progress; able to detect those lesions that are treatable or have prevented their progression with an intervention, and; have high sensitivity (few false negatives) and high positive predictive value. Stomatoscopy has great potential for the first four characteristics and has shown high sensitivity (few false-negatives) and a positive predicted value of 70.5%. Further studies are required to determine if it is a useful and practical examination for mass screening, or if it is suitable only for secondary centers of diagnostics.

The method is quick and easy to perform. Additionally, it may be useful in the clinical office, in the surgery room or even intraoperatively (in addition to freezing diagnosis), and others.

Conclusions

The results (accuracy, sensitivity, specificity, and positive

and negative predictive values) show that stomatoscopy, a non-invasive office-based diagnostic procedure, can be a useful test concerning the lesions of the lower lip vermilion area.

References

1. Scully C, Newman L, Bagan JV. The role of the dental team in preventing and diagnosing cancer: 3.oral cancer diagnosis and screening. Dent Update 2005; 32:326-37.
2. NCI - National Cancer Institute. Oral cancer. Available from: <www.cancer.gov/cancertopics/types/oral>. [2010 mar 12].
3. Ministério da Saúde. Instituto Nacional de Câncer. Estimativa/2008 Incidência de câncer no Brasil. Rio de Janeiro: INCA; 2007.
4. Holmes JD, Dierks EJ, Homer LD, Potter BE. Is detection of oral and oropharyngeal squamous cancer by a dental health care provider associated with a lower stage at diagnosis? J Oral Maxillofac Surg 2003; 61:285-91.
5. Mashberg A, Samit A. Early diagnosis of asymptomatic oral and oropharyngeal squamous cancers. CA Cancer J Clin 1995; 45:328-51.
6. Silverman Jr S. Early diagnosis of oral cancer. Cancer 1988; 62:1796-9.
7. Olivo M. Endoscopic fluorescence imaging to detect neoplasia in oral cavities. Cancer Update Nat Cancer Centre Singapore 2004; 3: (Serial online). Available from: <http://www.nccs.com.sg/pbcation/CU/vol3_04/p5-1.htm> [2010 Mar. 15]
8. Shedd D P, Hukill P B, Bahn S. In vivo staining properties of oral cancer. Amer J Surg 1965, 110:631-4.
9. Oliveira BV, Ramos GHA, Sampaio LA, Biasi L. Uso do colposcópio na avaliação de lesões da boca. Rev Bras Cir Cabeça Pescoço. 2007, 36:83-6.
10. Gynther GW, Rozell B, Heimdhal A. Direct oral microscopy and its value in diagnosing mucosal lesions: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90:164-70.
11. Hamou JE. Microhysteroscopy. Acta Endoscópica 1980; 10:415-22.
12. Hamou JE. Microendoscopy and contact endoscopy. Brevet Français 79; 04168 Paris, 1979; International Patent PCT/FR80/0024, Paris, 1980. US Patent 4.385.810. Washington, DC, 1983.
13. Andrea M, Dias O. Rigid and contact endoscopy associated to microlaryngeal surgery. Arq Portugueses ORL Pat Cerv-Fac, Lisboa [Suppl 2], 1994.
14. Andrea M, Dias O, Macor C, Santos A, Varandas J. Contact endoscopy of the nasal mucosa. Acta Otolaryngol 1997; 117:307-11.
15. Xiaoming H, Haiqiang M, Manquan D, Jianyong S, Kela L, Xiaoman L, et al. Examination of nasopharyngeal epithelium with contact endoscy. Acta Otolaryngol 2001; 121:98-102.
16. Pak MW, To KF, Lee JC, Liang EY, van Hasselt CA. In vivo real-time diagnosis of nasopharyngeal carcinoma using contact rhinoscopy. Head Neck 2005; 27:1008-13.
17. Romano FR, Voegels RL, Goto EY, Prado FA, Butugan O. Nasal contact endoscopy for the in vivo diagnosis of inverted schneiderian papilloma and unilateral inflammatory nasal polyps. Am J Rhinol 2007; 21:137-44.
18. Dedivitis RA, Guimarães AV. Contact endoscopy for intraoperative parathyroid identification. Ann Otol Rhinol Laryngol 2003; 112: 242-5.
19. L'Estrange P, Bevenius J, Williams L. Intraoral application of microcolpohysteroscopy: a new technique for clinical examination of oral tissues at high magnification. Oral Surg Oral Med Oral Pathol 1989; 67:282-5.
20. Pelucchi S, Bianchini C, Travagli M, Pastore A. Contact endoscopy of the oral mucosa: preliminary results. Acta Otorhinolaryngol Itálica 2007, 27:59-61.
21. Ramos GHA, Dedivitis RA, Padron C, Pedruzzi P, Oliveira BV. Câncer de boca e do lábio: microestomatoscopia. In: XX Congresso Brasileiro de Cirurgia de Cabeça e Pescoço, 03 a 06 de setembro de 2005, Salvador-BA. (abstract TL – 042)
22. Pak MW, To KF, Leung SF, van Hasselt CA. In vivo diagnosis of persistent and recurrent nasopharyngeal carcinoma by contact rhinoscopy. Laryngoscope 2002; 112:1459-66.
23. Loyola A, Valle E, Lopes LM. In: CPDT- Centro Pré-Natal de Diagnóstico e Tratamento: Microcolposcopia. Disponível em <http://www.cpdt.com.br/sys/interna.asp?id_secao=3&id_noticia=51> [2010 mar 12].
24. Parisi CMA. Microcolposcopia. Available from <http://www.carmeloparisi.it/microcolposcopia.html> [2010 mar 12].
25. Carriero E, Galli J, Fadda G, Di Girolamo S, Ottaviani F, Paludetti C. Preliminary experiences with contact endoscopy of the larynx. Eur Arch Otorhinolaryngol 2000; 257:68-71.
26. WHO Collaborating Centre for Oral Precancerous Lesions. Definition of leucoplakia and related lesions: an aid to studies on oral precancer. Oral Surg oral Med Oral Pathol 1978; 46:518-39.
27. Ramos G H A, Tavares M R, Dedivitis R A, França C M, Oliveira B V, Pedruzzi PA. Endoscopia de contato (microestomatoscopia) nas lesões da boca e do lábio: avaliação do método. Rev Col Bras Cir 2008; 35: 355-60.
28. Birdsong GG, Davey DD, Darragh TM, Elgert PA, Henry M. Amostra adequada. In: Salomon D, Nayar R, editores. Sistema Bethesda para citopatologia cervicovaginal. Rio de Janeiro: Revinter; 2005. p.1-11.
29. Ramos GHA, Dedivitis RA, Padron C, Pedruzzi P, Oliveira BV. Avaliação das lesões da cavidade oral por endoscopia de contato. Rev Bras Cir Cabeça Pescoço 2006; 35:85-7.
30. Gray MGL, Burls A, Elley K. The clinical como effectiveness of toluidine blue dye as an adjunct to oral cancer screening in general dental practice. A West Midlands Development and Evaluation Service Report; 2000. Avaliable from: < http://www.rep.bham.ac.uk/2000/toludine_blue.pdf> [2010 mar 22]
31. Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol 2008; 44:10-22.

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