Does autofluorescence help detect recurrent squamous cell carcinoma? A prospective clinical study

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Objective. In oral squamous cell carcinoma (OSCC), 20% of patients experience local recurrences. In this study, the addition of autofluorescence to a standard incandescent light examination was evaluated to enhance detection rates of recurrences in OSCC.
Study Design. Patients with OSCC who underwent follow-up examinations were included in this prospective cohort study. All patients (with or without recurrences) were examined clinically and with autofluorescence (using VELscope; Mectron, Cologne, Germany) and biopsy was used to examine suspect lesions for recurrences. Variables likely to influence results were analyzed. An analysis of dependencies, a general log-linear analysis, and a binary regression analysis were performed using SPSS version 26 (SPSS Inc., Chicago, IL).
Results. The study included 195 patients and in 39 cases a biopsy was performed. Results showed significantly more recurrences with the addition of autofluorescence to the usual clinical examination ($P \geq .5$). Sensitivity was 95.2% and specificity was 100%.
Conclusions. This study showed the advantages of adding autofluorescence to routine clinical assessments in OSCC follow-up examinations.
Clinical trial registration: German Clinical Trials Register DRKS-ID: DRKS00004836 (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:258−263)

Malignancies of the head and neck region occur at a rate of 3.4% in males and 1.3% in females. Ninety-five percent of those present as head and neck squamous cell carcinomas.[1] In patients with head and neck squamous cell carcinomas, the 5-year survival rate is less than 50%.[2] Even after initial therapy, one-fifth of patients suffer local recurrences, with 76% of recurrences occurring within the first 2 years after the primary diagnosis. Secondary tumors occur in up to 30% of cases.[3] Early and intense follow-up screenings are, therefore, crucial, especially because early forms of oral squamous cell carcinoma (OSCC) and recurrences develop without clinical symptoms.[4] Follow-up intervals vary, depending on the hospital involved, but should not exceed 3 months within the first 3 years and 6 months within 5 years of the primary diagnosis.[5] After-care investigations usually involve clinical assessments, including inspection and palpation of the head and neck region and assessment of symptoms, such as fatigue, fever, night sweats, and weight loss, and follow-up imaging (i.e., computed tomography [CT], magnetic resonance imaging, positron emission tomography/computed tomography [CT], cone beam computed tomography, and ultrasonography).[5] Precancerous lesions to look for in clinical assessments are leukoplakia, erythroplakia, lichen mucosae, and oral submucosal fibrosis.[6-8] Autofluorescence imaging has become an important tool in cancer detection. The VELscope (Visual Enhanced Lesion scope; Mectron, Cologne, Germany) has been used for decades as an adjunctive diagnostic device in the diagnosis of premalignant lesions.[9-11] It uses tissue autofluorescence (wavelength 400−460 nm) to detect abnormal areas showing loss of fluorescence.[12] As recurrences occur regularly and clinical evaluations are challenging, the use of autofluorescence may be beneficial in the detection of recurrences in OSCC. The purpose of this study was to determine whether supplementary technology, such as autofluorescence, improves the detection rates of recurrent OSCC. We hypothesized that autofluorescence enhances sensitivity and specificity in OSCC follow-up examinations. The specific aims of the study were to evaluate (1) if autofluorescence enhances

Statement of Clinical Relevance

The use of autofluorescence in follow-up examinations of patients suffering from squamous cell carcinoma was re-evaluated in this study. The most effective way of combating oral cancer and its recurrences is early detection, diagnosis, and eradication of early-stage lesions.

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Received for publication Aug 9, 2019; returned for revision Mar 30, 2020; accepted for publication Apr 20, 2020.

2212-4403/$-see front matter
https://doi.org/10.1016/j.oooo.2020.04.809

detection rates, (2) how much autofluorescence adds to sensitivity and specificity, and (3) if there are any variables, such as primary tumor location, type of reconstruction, inflammation, sex, smoking, and so on, which affect the predictor outcome of the autofluorescence assessment.

MATERIAL AND METHODS

Study design/sample

To address the research purpose, we designed and implemented a prospective clinical trial to evaluate the usefulness of adding autofluorescence to clinical examinations. The study population included all patients presenting to the Department of Craniomaxillofacial Surgery at the Hannover Medical School for evaluation and management of OSCC recurrences between April 2012 and May 2013.

To be included in the study sample, patients had to have a history of oral cancer, routinely attend follow-up examinations, and consent to participation. Patients were excluded if they were taking part in other clinical studies, if they might be pregnant, if they were minors, if they had a history of substance abuse, if they were handicapped, or if they were under legal supervision.

Variables

The outcome variable was to determine if autofluorescence enhances detection rates of recurrent OSCC. Different levels of examinations were evaluated. First, only white light examination was performed (variable 1 = conventional oral examination [CA]). Second, in addition to the clinical examination, autofluorescent light examination (AF) was performed (variable 2 = clinical examination + autofluorescence). Moreover, factors that may be related to the outcome were determined and compared: sex, age, smoking (y/n), alcohol (y/n), inflammation (analyzed by using additional diascopy), burning sensation of the tongue (TB; y/n), localization of primary tumor (as described in the surgical protocol), localization of efflorescence (according to the Roed-Petersen classification[13]), time between follow-up examination and primary tumor therapy (median in months), and type of reconstruction (as described in the surgical protocol).

Data collection methods

All patients were seen and examined by the same experienced maxillofacial surgeon. In addition to the usual routine clinical examination according to the standard protocol,[14] the Roed-Petersen protocol[13] was also completed. Then, in all patients, the oral cavity was examined using VELscope (Mectron, Cologne, Germany) and a Pentax reflex camera. To ensure perfect conditions, the examinations took place in a separate room with dimmed lights. Safety goggles were worn by the patients and the examiner throughout the inspection. The examination followed the standard protocol, and every suspect region was again marked according to the Roed-Petersen protocol. A suspect finding in the VELscope assessment was defined as loss of fluorescence (Figure 1). To detect inflammation, additional diascopy was performed. In case of a suspect lesion, either in the clinical or in the VELscope assessment, it was up to the examining maxillofacial surgeon to take all clinical aspects into account (e.g. inflammation) to determine whether additional biopsy was necessary.

Data analysis

To obtain significant data, the ideal sample size was calculated using G*Power version 3.1. (2014) (Heinrich-Heine-University, Duesseldorf, Germany) and was set to a number of at least 186 patients, including 18 patients suffering from recurrences of malignancies. Data analysis was performed using SPSS statistical software for Mac version 26 (SPSS Inc., Chicago, IL) and Microsoft Excel version 16.16.3 (Microsoft Corp., Redmond, WA). Means and standard deviations (mean ± standard deviation) were evaluated. An analysis of dependencies was performed using the χ^2 test for associations. This was conducted for all study variables and biopsy outcomes. A 3-way log-linear analysis was performed to determine a hierarchical unsaturated model of the associations among the variables. Furthermore, a binomial logistic regression analysis was performed to ascertain the effects of the associated variables based on the likelihood of participants' biopsy results being positive.

RESULTS

In this study, 195 patients (age range 30−93 years; 37.9% females and 62.1% males) were examined. Of these, 174 patients were recurrence-free, and recurrences were diagnosed in 21 patients. The primary therapy for all patients was surgery. In total, 73 patients presented with suspect lesions (Figure 2): 24.7% (n = 18) of primary lesions and 19% (n = 4) of later recurrences were located at the gingiva; 13.7% (n = 10) at the dorsum of the tongue; 15.1% at the vestibulum; and 19.2% at the mouth base. Of those identified in the mouth base, 33.3% (n = 7) were later recurrences. In the VELscope assessment alone, 62 patients were identified to have suspect lesions. In all cases, additional diascopy was performed, and inflammation was detected in 14 cases (22.6%). These patients were seen again after 14 days. All of the 14 patients had inflammatory lesions. In 26 cases, the lesions detected with the use of VELscope were also identified during clinical examination. In 39 patients, additional biopsy was performed, and 21 (11 females, 10 males) turned out to be positive for recurrence of the malignancy. Of these

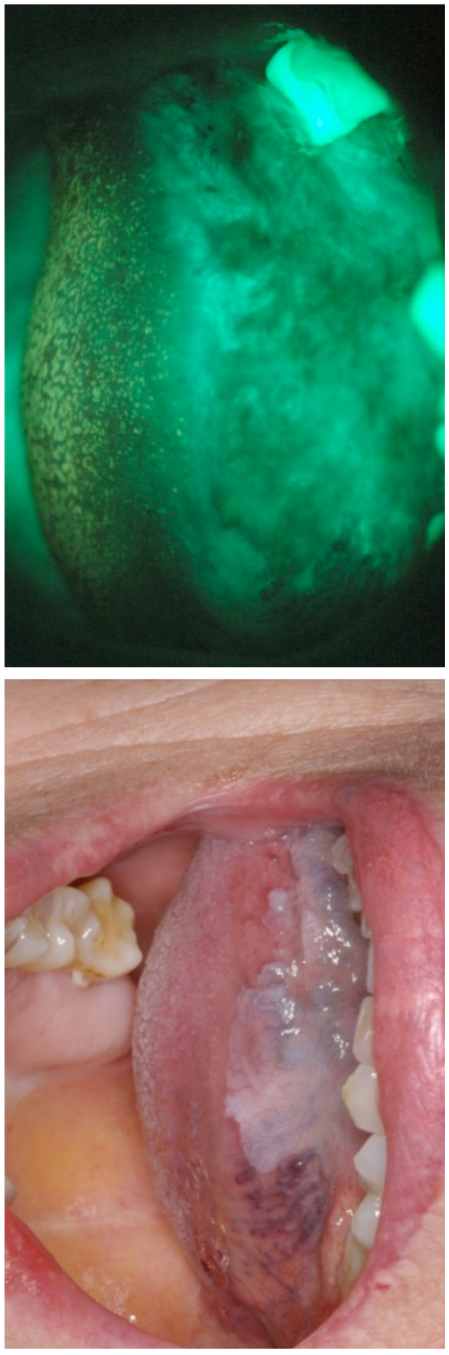

Fig. 1. Demonstrates a suspect lesion in the standard clinical white-light examination *(left)*. It shows an around 3 × 2 cm large leucoplakia on the lateral tongue. In the VELscope assessment *(right)*, lesions are identified by loss of fluorescence in comparison with the unaffected tongue.

21 patients, 20 showed suspect lesions in the incandescent light examination, whereas all 21 showed loss of fluorescence in the VELscope assessment. The interval between primary tumor therapy and follow-up examination was 3 to 12 months in 23.6%, 12 to 96 months in 73.8%, and 97 to 108 months in 2.6%.

Analysis of dependencies

A χ^2 test for association was conducted for all study variables and biopsy results. Not all expected cell frequencies were greater than 5. There were 4 statistically significant associations between study variables and biopsy results (Table I). A 3-way log-linear analysis was performed to determine a hierarchical unsaturated model for the associations among the variables, as shown in Table I. Of the participants, 195 showed not all 8 cells having greater than 5 expected frequencies, no outliers, and approximately normally distributed adjusted residuals for the chosen model. An unsaturated model was chosen using the SPSS hierarchical log-linear model selection procedure with a backward-elimination stepwise procedure. This produced a model that included all main effects and three 2-way associations between biopsy results and clinical incandescent light examinations, between biopsy results and autofluorescence examination, and between biopsy results and TB. Only one main effect of sex stayed significant in the analyses. The model had a likelihood ratio of $\chi^2(2) = 0.816$; $P = .665$. The log-linear parameter estimations are presented in Table II. Furthermore, a binomial logistic regression analysis was performed to ascertain the effects of age, sex, and CA, AF, and TB on the likelihood of the participants having positive biopsy results (Table III). No continuous variable was included in the test. Linearity of the continuous variables with respect to the logit of the dependent variable was not necessary for the assessment. The logistic regression model was statistically significant, $\chi^2(2) = 105.667$; $P < .001$. The model explained 93.5% (Nagelkerke R2) of the variance in biopsy results and correctly classified 99.4% of cases. The accuracy of the explained variance is enhanced with the addition of autofluorescence by approximately 10% to 12%. Sensitivity was 95.2%, specificity was 100%, positive predictive value was 100% and negative predictive value was 99.27%. The area under the receiver operating characteristic curve was 0.997; the 95% confidence interval was 0.990 to 1.00, which is an excellent level of discrimination.[15]

DISCUSSION

Despite advances in technology and thus better and faster primary and adjuvant therapies, 5-year survival rates, especially for patients with advanced oral cancer, have only slightly improved over the last decades.[16]

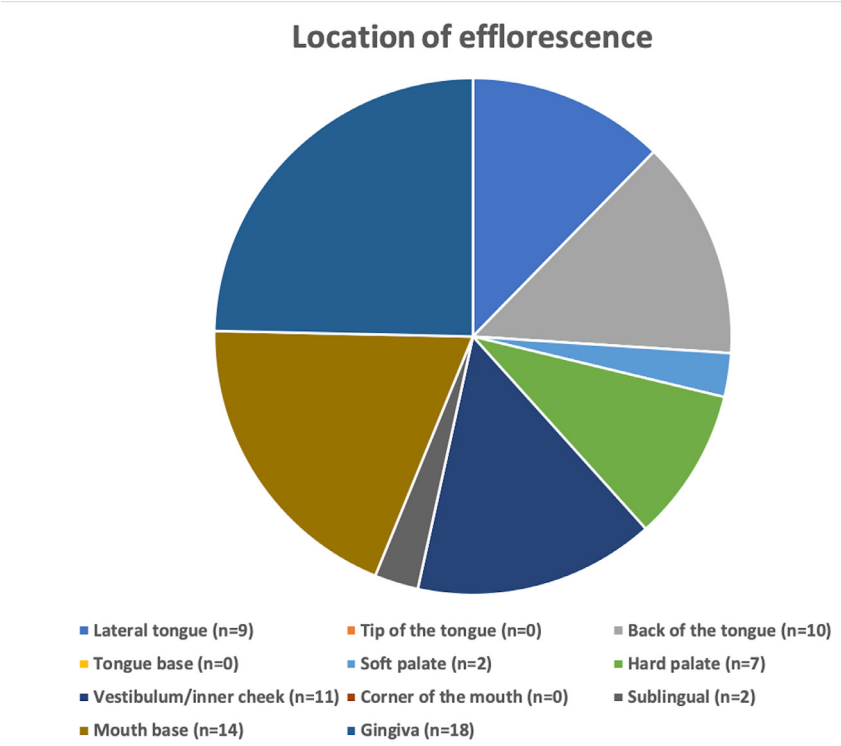

Fig. 2. Locations where efflorescences were found during the standard white-light clinical examination (n = 73). Over 50% could be found on the lateral tongue, the mouth base, and the gingiva.

Consequently, the most effective way of combating oral cancer and its recurrences is early detection, diagnosis, and eradication of early-stage lesions and their precursors.[17] This study evaluated the use of autofluorescence in the detection of recurrences of OSCC. It was hypothesized that additional technology enhances the predictability of routine clinical examinations with regard to recurrent OSCC. The findings of this study show that the addition of autofluorescence to standard examinations does enhance the predictability of a positive biopsy result with approximately 10% to 12% accuracy.

Furthermore, the effect on likely variables, such as sex, age, smoking, alcohol use, inflammation, TB, localization of primary tumor, localization of efflorescence, interval between follow-up examination and primary tumor therapy, and type of reconstruction on the VELscope assessment, were evaluated. Surprisingly, only sex and TB affected the outcome significantly.

Table I. Significant associations between biopsy outcomes and study variables in the χ^2 test

Variable	χ^2	*P value*
Clinical white-light assessment (CA)	133.61	$P \leq .01$
Autofluorescence assessment (AF)	30.38	$P \leq .01$
Sex (G)	6.61	$P = .01$
Burning tongue sensation (BT)	5.19	$P = .015$

The addition of autofluorescence was more effective in males than in females in predicting a positive result on biopsy. The reason could be that women are more likely to suffer from autoimmune diseases related to their vulnerable mucosa[18] or the more fragile or thinner mucosa, in general, leading to false-positive results. The effect of TB on VELscope predictability might be explained by autoimmune disease and/or by inflammation, which leads to increased cell destruction and thus loss of fluorescence. Inflammation, which was tested by using diascopy, showed no significant effect on the VELscope assessment.

Table II. Partial likelihood ratio: significant results of the χ^2test

Variables	*df*	*Partial* χ^2	*Significance*
CA* B	1	74.861	< .001
AF*B	1	8.451	.004
CA	1	91.502	<.001
AF	1	26.684	<.001
B	1	95.199	<.001
TB	1	38.116	<.001

AF, autofluorescence assessment; *B,* results of biopsy *CA,* clinical incandescent light assessment; *df,* degrees for freedom; *TB,* burning tongue sensation.

* It indicates that the results of the clinical incandescent light assessment (CA) and the results of the biopsy (B) have been used for the calculation. Same for AF and B.

Table III. Logistic regression predicting likelihood of a positive (B = 1) or negative (B = 0) biopsy result based on clinical incandescent light assessment (CA) and autofluorescence assessment (AF)

Parameter	*Estimate*	*Standard error*	*Z*	*Significance*	*95% confidence interval*	
					Lower bound	*Upper bound*
Constant	2.784	.247	11.255	<.001	2.300	3.269
CA = 0 * B = 0	0.602	.308	1.957	.050	−0.001	1.205
CA = 0 * B = 1	−2.996	1.025	−2.924	.003	−5.004	−0.987
CA = 1 * B = 0	−3.610	.766	−4.711	<.001	−5.112	−2.108
AF = 0 * B = 0	1.272	.207	6.155	<.001	.867	1.677
AF = 0 * B = 1	−1.447	.556	−2.604	.009	−2.536	−.358

CA was positive when a suspect lesion was seen (negative CA = 0, positive CA = 1), a loss of fluorescents was counted as a positive autofluorescence result (negative AF = 0; positive AF = 1). Further parameters were left out because they were redundant.
Model: Poisson.
Design: Constant + (CA * B) + (AF * B) + CA + AF + B.
AF, autofluorescence assessment; *B,* results of biopsy *CA,* clinical incandescent light assessment.
* It indicates that the results of the clinical incandescent light assessment (CA) and the results of the biopsy (B) have been used for the calculation. Same for AF and B.

Follow-up OSCC assessments almost entirely depend on the surgeon performing the examinations.[14] Recurrences usually are nonsymptomatic and diagnosis is made more difficult by mucosal changes related to surgical reconstruction and adjuvant radiotherapy and chemotherapy.[19] Even experienced clinicians are at risk of not recognizing suspect lesions, resulting in severe consequences for the individual patient.[4] Because of subjectivity, distinct rates of specificity and sensitivity of clinical examinations cannot be provided in the literature. In this study, clinical examinations showed 88.9% accuracy in predicting the results of biopsy. Diagnostic decisions should not rely only on the examiner's expertise. CT/magnetic resonance imaging, cone beam computed tomography, positron emission tomography/CT, or ultrasonography support diagnostics but cannot be performed in every single follow-up assessment.[5] VELscope could be an easy, quick, cheap, and safe option to enhance objectivity in follow-up examinations. Because the results of VELscope have to be interpreted by the examiner, assessments are still prone to subjectivity. Therefore, the examiner has to be trained in its use and interpretation. In 2016, Scheer et al. tested VELscope for its use in the detection of recurrences. In their study, the use of VELscope alone was tested against the diagnostic properties of biopsy in 41 patients with recurrent OSCC. Their results for sensitivity and specificity were 33.3% and 88.6%, respectively, for the VELscope examination to identify malignant oral lesions with autofluorescence. Scheer et al., therefore, concluded that VELscope provided no additional value in follow-up examinations.[20] However, biopsy is only performed if recurrences present as clinically obvious lesions. In this study, 195 patients were evaluated. All of them were assessed through clinical examination and the VELscope device. Use of the clinical examination alone was compared with the combination of clinical and VELscope examinations. Suspect lesions were reassessed through biopsy. Clinical examination in combination with VELscope evaluation showed sensitivity of 95.2% and specificity of 100%. The likelihood of a positive biopsy result was enhanced from 88.9% to 99.27% with the addition of autofluorescence. A pilot study by Lane et al. showed sensitivity of 98% and specificity of 100%, similar to the results obtained by using autofluorescence in patients with malignancies in previous studies.[12] In contrast, Sweeny et al. could only show sensitivity of 81% and specificity of 50%.[21] In this study, additional diascopy was performed to reduce false-positive results by excluding inflammation. A common disadvantage of VELscope is that it cannot distinguish between benign and malignant lesions. Therefore, invasive biopsy is unavoidable, with the possibility of the primary diagnosis leading to unnecessary treatment.[22-25] This may not be as much of a problem in follow-up assessments because the lesion is more likely to be a recurrence than a newly acquired efflorescence. Nevertheless, false-positive results can have a strong psychological impact on patients.[26] Recurrences are far more likely to occur within the first 2 to 3 years after primary tumor therapy.[3] Therefore, we examined the relevance of the follow-up interval to the outcomes of the VELscope assessment. The interval between primary tumor diagnosis and follow-up examination ranged from 3 months to 108 months. No significant correlation could be detected. The number of biopsies (n = 39) was compared to to the total number of cases examined. Only 21 positive biopsy results were found. If more cases were included, more reliable results could have been obtained, and further dependencies of variables might have been discovered. This limits the significance of this study. Unfortunately, no differences between patients' adjuvant therapies could

be identified. It would have been interesting to see whether monotherapy with chemotherapy, radiation, or surgery affected the results. The use of VELscope alone is not enough to detect recurrences. Outcomes show that additional VELscope examinations increase the predictability of recurrent OSCC by 10% to 12%. Our results also showed that 88.9% of cases can be diagnosed using clinical assessment alone. To avoid false-negative or false-positive results, VELscope examination should always be accompanied by clinical examination and diascopy.

CONCLUSIONS

Despite its limitations, this prospective clinical cohort study showed improved detection of recurrent OSCC with the use of autofluorescence in addition to routine clinical assessments. The effect of adjuvant therapy still needs to be investigated and further technical advances are needed to improve the reliability of additional diagnostic methods.

ACKNOWLEDGMENTS

We thank Mr. Erik Riedel for his help with the statistical analysis in this study.

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