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Oral premalignant lesions: is a biopsy reliable?

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Abstract

PURPOSE: The purpose of the present retrospective study was to learn whether a biopsy of oral premalignant lesions, leukoplakia and erythroplakia, shows histopathological findings representative of the whole surgically removed lesion. Moreover, to see whether histopathological characteristics of the whole lesion are significant for future malignant development after surgery.

MATERIALS AND METHODS: A total of 101 lesions in 96 patients were included, 42 lesions (41%) being homogenous and 50 (50%) non-homogenous leukoplakias, whereas nine (9%) were erythroplakias. The lesions were biopsied and subsequently surgically removed on the average of 10.4 months after biopsy. Surgical specimens were examined in two or more step sections distributed throughout the specimen. The histological findings of the biopsies were compared with those of the whole lesions. After surgical intervention the patients were followed (mean 6.8 years, range: 1.5–18.6), and new biopsies taken in case of recurrences. Smokers (73%) were encouraged to quit smoking and candidal infections were treated. The possible influence of different variables on the risk of malignant development was estimated by means of logistic regression analysis.

RESULTS: Histological examination of the whole lesions showed that seven lesions (7%) harboured a carcinoma and 70 lesions (69%) showed a degree of epithelial dysplasia or carcinoma in situ. Eleven lesions (12%) developed carcinoma after a mean follow-up period of 7.5 years. A comparison of the degree of dysplasia in the biopsies with that of the whole lesion demonstrated variation with concurrent diagnosis in 49% of the lesions and in 79% after inclusion of lesions with one degree up or down the scale of epithelial dysplasia.

CONCLUSION: The estimated odds ratio showed that none of the associated variables including presence of any degree of epithelial dysplasia in the whole lesion, site, demarcation and smoking had influence on the risk of malignant development.

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Introduction

The main purpose of identifying oral premalignant lesions is to prevent malignant transformation by initiating adequate intervention, and it is widely approved that the type of intervention should be based on histopathological features of a biopsy of the lesions. This is due to an important paradigm about premalignancy: the presence and grade of epithelial dysplasia plays a significant role for future malignant development (1–9). Consequently, the presence of epithelial dysplasia in a biopsy usually results in a more aggressive approach than in case of no dysplasia. The significance of epithelial dysplasia in predicting risk of future malignant development has been questioned in the past (3, 7, 8, 10–16) and based on a 7-year follow-up study of 269 premalignant lesions we have recently shown that presence of epithelial dysplasia in the biopsies was not a significant factor for malignant development in lesions with surgical or no surgical intervention (17). Two aspects, however, are important to understand whether a biopsy is in fact reliable. One is the well-known interobserver and intraobserver variation in reading the degree of epithelial dysplasia, which has been addressed previously (10, 12, 15, 18). Another aspect is whether the histological diagnosis of a biopsy reflects the true nature of the lesion in question, i.e. whether the biopsy is representative of the whole lesion and whether the result of treatment depends on the histopathological findings of the whole lesion rather than those of the biopsy, which usually determines the treatment regimen.

To understand the insignificance for future malignant development of epithelial dysplasia in biopsies, the hypothesis behind the present study was that biopsies are not representative of the whole premalignant lesion and that the outcome after follow up of oral premalignant lesion depends on histopathological features of the whole lesion.

Therefore, the aim of the present study was to learn whether biopsies of premalignant lesions taken by experienced oral surgeons were representative of the whole lesion. Moreover, it was the aim to learn whether the long-term outcome after surgical removal of oral premalignant lesions was related to the presence of epithelial dysplasia as revealed by histopathological examination of the whole lesion.

Materials and methods

Lesions

In this retrospective study of surgically removed premalignant lesions, part of which has been reported previously (17), a total of 101 lesions comprising 42 (42%) homogenous, 50 (50%) non-homogenous leukoplakias, and nine (9%) erythroplakias in a total of 96 patients (52 women and 44 men; mean age: 61 years, range: 23–92), referred between 1977 and 1997, were included. The clinical diagnoses of the lesions were based on the criteria provided by Axéll et al. (19), adjusted to the most recent definition (20), adopted by WHO (21). The criteria for surgical treatment was that biopsies of the lesions exhibited epithelial dysplasia and/or the lesions were located on the lateral margin or the ventral surface of the tongue or in the sublingual region (17).

The histopathological diagnosis of epithelial dysplasia was made according to WHO definitions (21), and the diagnosis of the biopsy was compared with that of the whole lesion after surgical removal which took place on the average of 10.4 months after the biopsy. Surgical specimens were examined in two or more step sections distributed throughout the specimen; the number of step sections (1–10) depending on the size of the specimens. The histopathological examination showed carcinoma in seven of the surgically removed lesions. The patients with carcinomas were referred to the Department of Oncology for further treatment, and the remaining 94 lesions were described and followed as previously reported (17). The mean follow-up period was 6.8 years the range being 1.5–18.6. The clinical set-up included antimycotic treatment of lesions with *Candida* infection before or after surgical removal as demonstrated by the presence of hyphae or pseudohyphae in periodic acid-Schiff-stained sections or smears from the lesions. The patients were treated with amphotericin B, or miconazole for 4–6 weeks. Patients were informed about the premalignant nature of the lesions and smokers (73%) were encouraged to quit smoking throughout the entire follow-up period.

Statistical data

Analysis of data were performed using the package SAS (version 8.02, SAS Institute, Inc., Cary, NC, USA). The possible role of different factors for malignant development of the premalignant lesions was estimated by means of logistic regression analysis. The factors were incorporated as independent variables in the analysis if they had an association with the outcome variable (malignant transformation) at a $P < 0.20$. The included independent variables are shown in Table 3. With the logistic regression analysis the odds ratio and the corresponding confidence intervals were calculated. Ordinary level of significance was 0.05.

A test for trend was performed to analyse the occurrence of carcinomas in the various groups of epithelial dysplasia.

Results

The histopathological characteristics of the total lesions after surgery are illustrated in Fig. 1. As mentioned above, seven carcinomas, which were not found in the biopsies, were revealed in the lesions, and three of these lesions were characterized as homogenous and four as non-homogenous leukoplakias.

The histopathological characteristics of the biopsies vs. those of the whole lesions are shown in Table 1. Whereas the histopathological examination of the total lesions showed a degree of epithelial dysplasia or carcinoma in situ in 70 lesions (69%) similar findings in the biopsies were seen in 72 lesions (71%). A comparison of the degree of dysplasia in the biopsies with that of the whole lesion; however, demonstrates variation and concurrent diagnosis was found in 49% of the lesions and in 79% after inclusion of lesions with one degree up or down the scale of classifying epithelial dysplasia and carcinoma in situ (21). Whereas a more severe diagnosis of the total lesion than of the biopsy, i.e. underdiagnosis in the biopsy was made in 35% of the lesions, a less severe diagnosis of the total lesion in the biopsy was made in 17%. Importantly, however, lesions, which in the biopsies had shown no, slight or moderate dysplasia, harboured carcinomas in 8% of the cases. On the other hand, lesions which in the biopsies showed severe dysplasia or carcinoma in situ never revealed a carcinoma in the total lesion. The duration between biopsy and surgical removal for lesions with initial carcinoma was shorter (mean: 4.4 months) than for those without (mean: 10.8 months, $P = 0.0002$).

Table 1 Histopathology of biopsy vs. total lesion

		Total lesion						
		No dysplasia	Slight dysplasia	Moderate dysplasia	Severe dysplasia	Carcinoma In situ	Carcinoma	Total
Biopsy	No dysplasia	18	3	4	2	0	2	29
	Slight dysplasia	3	14	9	2	0	2	30
	Moderate dysplasia	2	5	8	6	1	3	25
	Severe dysplasia	1	2	2	5	1	0	11
	Carcinoma In situ	0	0	0	2	4	0	6
	Total	24	24	23	17	6	7	101

Orange area indicates underdiagnosis in biopsies. Yellow area indicates overdiagnosis in biopsies. Red numbers indicate similar diagnosis in biopsy and total lesion.

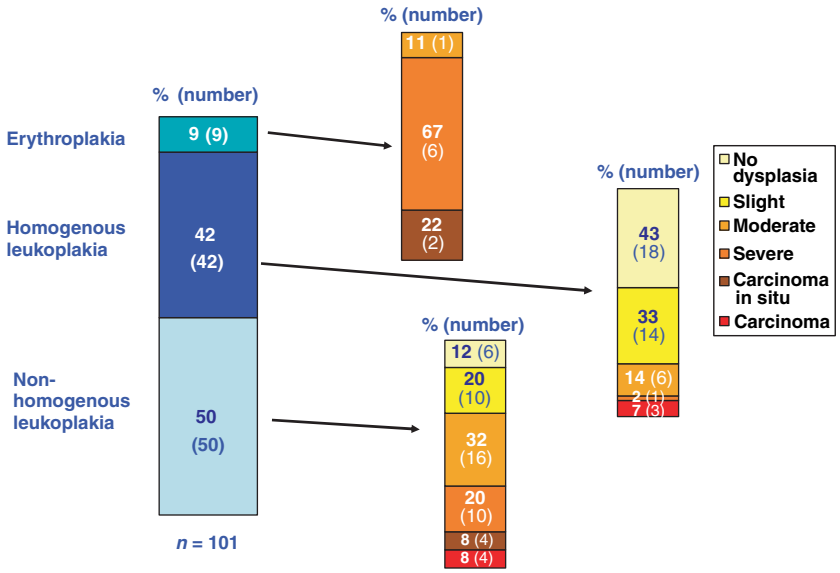

Figure 1 Presence of epithelial dysplasia, carcinoma in situ and carcinoma in total lesions by clinical type of lesion.

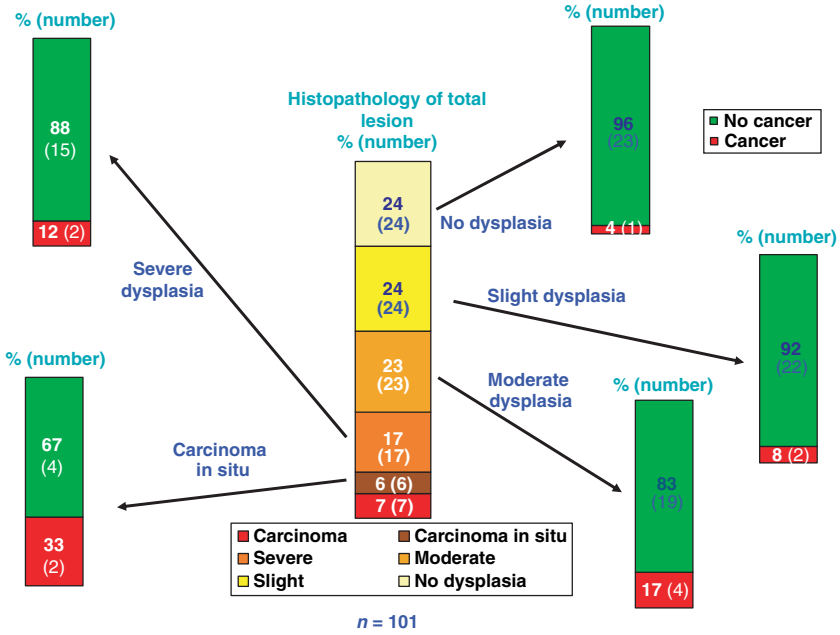

Figure 2 Cancer development after surgical intervention and follow up by degree of dysplasia and carcinoma in situ in total lesion.

As previously reported (17), 11 of the 94 surgically removed lesions (12%) developed carcinoma after a mean follow-up period of 7.5 years. The frequency of malignant development after surgical removal and follow up (Fig. 2) varied from 4% to 33% between groups with different degrees of epithelial dysplasia and carcinoma in situ in the total lesion. The highest frequency (33%) was found in the group of six lesions with carcinoma in situ. The distribution of developing carcinomas by degree of epithelial dysplasia and carcinoma in situ in the total lesion might be interpreted as a tendency of increased malignant development with increased degrees of epithelial dysplasia, but a test for trend showed that it was not statistically significant. The follow-up period of the groups with different degrees of epithelial dysplasia and carcinoma in situ (Table 2) showed no statistically significant difference ($P = 0.5$).

Only the variables in Table 3 fulfilled the inclusion criteria of the logistic regression analysis, which showed that none of the corresponding odds ratio was statistically significant.

Discussion

In a previous study we have demonstrated that the development of cancer in the oral premalignant lesions,

Table 2 Length of follow-up period by degree of epithelial dysplasia in total lesions

	Mean (years)	*N*
No dysplasia	7.6	24
Slight dysplasia	7.2	24
Moderate dysplasia	5.9	23
Severe dysplasia	7.2	17
Carcinoma in situ	6.3	6
Total	6.8	94

Table 3 Odds ratio estimates for carcinoma to occur ($N = 94$)

	Variable	*Point estimate*	*95% confidence limits*	
Clinical type	2 vs. 3	4.9	0.5	51.6
Size	1 vs. 2	4.5	0.8	24.8
Histology	1 vs. 3	3.6	0.2	68.9
Histology	2 vs. 3	1.9	0.2	19.3
Clinical type	1 vs. 3	1.8	0.1	43.8
Tobacco habit	1 vs. 2	1.6	0.3	8.1
Border	1 vs. 2	0.5	0.1	2.3

Clinical type: 1. erythroplakia, 2. non-homogenous leukoplakia, 3. homogenous leukoplakia; size: 1. $\geq$200 mm^2, 2. $<$200 mm^2; histology: 1. carcinoma in situ, 2. slight, moderate or severe dysplasia, 3. no dysplasia; tobacco habit: 1. smokers at the first examination, 2. non-smokers at the first examination; border: 1. sharp demarcation, 2. diffuse demarcation.

leukoplakia and erythroplakia, was dependent on the clinical type of lesion and size, whereas it was independent of surgical removal, site, smoking, demarcation of lesions and presence or absence of epithelial dysplasia in a pre-surgical biopsy (17). The lack of significance for future malignant development of epithelial dysplasia in a pre-surgical biopsy may be because the reading of epithelial dysplasia is subjective, because the biopsy taken is not representative of the whole lesion, or because epithelial dysplasia itself is not a significant prognostic factor for future malignant development.

In the daily work the clinician is faced with the problem of interobserver and intraobserver variation in reading the degree of epithelial dysplasia, which usually is not reflected in the histopathological diagnosis submitted. This problem is not addressed in the present study, but has been described previously (12, 15, 18, 22). Based on routine clinical work the present study describes whether the delivered histological diagnosis of a biopsy reflects the true nature of the lesion in question, i.e. whether the biopsy is representative of the whole lesion and whether the result of treatment depends on the histopathological findings of the whole lesion rather than those of the biopsy.

The histopathology of the whole lesions after surgical removal and stepwise sectioning revealed seven unexpected carcinomas (7%). This is important and the problem is why these carcinomas were not encountered in the biopsies. Were they there by the time of biopsy or did they occur in the period between biopsy and surgical intervention? Although the biopsies were taken by experienced oral surgeons, the question is impossible to answer, but obviously the surgeons have chosen the clinically most suspect area for biopsy. Moreover, it cannot be excluded that the carcinomas were induced by the incision as reported in an experimental study by Maeda and Kameyama (23) and previously discussed (17).

The discrepancies in the diagnosis of epithelial dysplasia in the biopsies vs. the whole lesions with only 49% agreement are not surprising considering the well-known subjectivity in the histopathological evaluation (10, 12, 15, 18, 22), the time span between biopsy and surgery (mean: 10.4 months) and the fact that the tissue changes may vary within the lesions. It was interesting that as much as 79% of the lesions had the same diagnosis or one degree up or down the scale of classifying epithelial dysplasia. In the light of a recent study reporting on underdiagnosis of premalignant lesions in biopsies (18), it was also notable that the relative number of lesions, which in the present study were underdiagnosed in the biopsies, was the double (35%) of the number of lesions which had a more severe diagnosis in the biopsies (17%). These figures show a non-random variation in the diagnoses, and this may reflect that with time the lesions are more prone to change towards malignancy than the opposite way.

A mean transformation time for dysplastic lesions to carcinoma of 33.6 months has been reported previously (6), and the present time interval with a mean of 10.4 months is far less, why time may not explain the underdiagnosis of carcinomas in the biopsies. Moreover, the distribution of developing carcinomas by degree of epithelial dysplasia and carcinoma in situ in the total lesion showed no significant tendency of increased malignant development with increased degrees of epithelial dysplasia. Various frequencies of developing carcinomas in these groups could be explained by different follow-up periods of the groups, but as shown above, there was no such difference.

Moreover, the logistic regression analysis showed that the different variables, including presence or absence of epithelial dysplasia, were insignificant and therefore appeared to have no influence on the course of the lesions in any of the groups examined. The number of observations in the various groups is limited and the findings, therefore, should be interpreted with caution. The histological features of the whole lesions, on the other hand, did not explain the lack of correlation between histological features of the biopsy and the future development of malignancy.

Most likely, recent findings of genetically altered epithelial cells unrevealed by routine histological examination and even in areas with normal histology may account for the lack of correlation (22). This, in addition to field cancerization may also result in unrevealed incomplete resection resulting in recurrence of lesions or development of frank carcinoma from residual genetically altered cells (24–26). The latter explanation for some of the present cases is supported by the finding that among 12 recurring lesions four later developed carcinoma (17). The use of molecular markers, including

TP53-mutated DNA, as supplementary indicators of a lesion's prognosis may be valuable for an improved result of treatment and follow up of the lesions (26).

In conclusion, the present study has shown that biopsies of premalignant lesions may not be reliable and that 35% of the total lesions had a more severe histopathological diagnosis, including 7% carcinomas, of the whole lesion compared with that of the biopsy taken on the average of 10.4 months previously. This is why the lesions should be followed by observations at close intervals (every 3–6 months) independent of presence or absence of epithelial dysplasia. Moreover, the course of premalignant lesions after surgical removal in the present study did not appear to be significantly associated with histopathological features of the total lesion. The present findings, therefore, emphasize the need for other tools for prediction of cancer development in susceptible lesions.

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