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Review

Biomarkers in dysplasia of the oral cavity: A systematic review

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SUMMARY

Oral dysplasia is a potentially precancerous lesion diagnosed histologically. While the risk of progression is associated with histological grade, it is currently impossible to predict accurately which lesions will progress. More accurate markers predicting progression to cancer would enable the targeting of these lesions for more aggressive treatment and closer follow-up. We have performed a systematic review with pooling of data to assess the evidence for the use of biomarkers in predicting transformation of oral dysplasia into cancer. We systematically searched the Cochrane library, MEDLINE, EMBASE, AMED, Cinahl and the Kings Fund electronic databases using the terms: oral dysplasia, leukoplakia, erythroplakia, biomarkers and genetic markers. The following *a priori* selection criteria were used: longitudinal cohort or case-controlled studies of oral dysplasia that progressed to cancer. Cross-sectional studies and studies reporting only on leukoplakia were excluded. Data were extracted by two reviewers. Quality assessment was carried out using validated tools. We assessed the relative risk of progression form oral dysplasia to cancer and pooled data where possible. 2550 studies were identified, from which 288 were scrutinised in greater detail. Of these, 247 were excluded, mainly due to cross-sectional design. Of the 41 studies containing follow-up data, 28 were excluded, most commonly due to data only being available for lesions once they had progressed to cancer. A lack of clear histological definition of oral lesions was also a common finding. Data were extracted from 13 longitudinal studies. The evidence consists mainly of small, single centre, retrospective studies. In oral dysplasia, loss of heterozygosity (LOH), particularly at the 3p ± 9p loci, increases the risk of progression to cancer (RR 17.60 (2.77, 108.37) $p < 0.001$), as does survivin (RR 30 (4.25, 197.73), $p \leqslant 0.001$), matrix metalloproteinase (MMP 9), (RR 19.00 (1.56, 209.38) $p = 0.02$) and DNA content (RR 12.00 (1.17, 82.10) $p = 0.03$). Other markers identified by this review including p53, p73, MMP 1 and 2 and cathepsin L mRNA, did not predict progression. LOH, survivin, MMP 9 and DNA content are potential markers for increased risk of progression from oral dysplasia to cancer. Many methodological limitations have been identified by this review, however, and we recommend these results are interpreted with caution. Research into this field should concentrate on longitudinal design, with pooling of data from multiple centres to achieve larger cohorts. We recommend standardisation of definitions to allow appropriate comparisons to be made.

Background

Oral dysplasia is a relatively common precursor of oral cancer. Progression to cancer varies widely ranging from 6% to 36%.[1] Histological grade is currently the best predictor of progression to cancer and provides the basis on which clinical decisions are made. The grade of dysplasia is determined by the degree of cellular abnormality above the epithelial basement membrane as originally defined by the World Health Organisation (WHO).[2]

The most recent WHO publication indicates that there is no clear consensus on the most clinically appropriate grading system for oral dysplasia.[3] Many factors play a part in this. Accuracy of grading is dependant on the quality of tissue and the site at which a biopsy is taken. Dysplasia grading is also subjective, with inter and intra-rater variability.[4,5] Furthermore, some lower grades of dysplasia progress to cancer whilst other, higher grades, remain static or even regress, irrespective of environmental factors.[6] A better system for the prediction of cancer progression is therefore needed.

Objectives

This systematic review examines the current evidence for the effectiveness of biomarkers and genetic abnormalities in predicting progression to cancer in patients with oral dysplasia.

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Methods

Search strategy

Our search strategy was developed in accordance with guidelines outlined in the Cochrane handbook for systematic reviews.[7] We searched and identified articles using the Cochrane library (1995-November 2007), MEDLINE (1950-November 2007), EMBASE (1974-November 2007), AMED (1985-November 2007), Cinahl (1982-November 2007) and the Kings Fund (1979-November 2007). The search was widened to include the Internet and handsearched reference lists of identified articles. We consulted experts within the field for further identification of relevant material. No language restrictions were imposed on the initial search.

In order to maximise identification of relevant articles we used the key phrase 'oral dysplasia' as our initial search term. In a second search, the keywords 'oral dysplasia,' 'leukoplakia' and 'erythroplakia' were individually cross referenced with the terms 'biomarkers,' 'genetic markers' and 'molecular markers.'

Selection criteria

Article titles and abstracts were reviewed and irrelevant papers were excluded (Fig. 1). If the abstract was deemed relevant then the full paper was reviewed for suitability by two researchers (JS, TR). If there was disagreement on the inclusion of a study then a third reviewer (HM) was consulted.

We limited selection to human studies of oral dysplasia defined on standardised histological assessment as outlined by the WHO.[2] Studies including oral lesions defined clinically, such as leukoplakia and erythroplakia, were excluded unless data on dysplasia were reported separately and could be extracted from published tables. We included all longitudinal studies that presented data for progressing and non-progressing oral dysplasias. These included case-control and cohort studies as well as consecutive case series. Both prospective and retrospective studies were included. Progressing lesions were defined as those dysplasias that developed cancer at the same site as the initial biopsy when followed over time. Non-progressing lesions were those matched comparisons followed for a similar or longer period that did not progress to cancer.

Cross-sectional studies of biomarkers in oral dysplasia were included in descriptive analysis only and excluded from prognostic analyses. Studies including cases where cancer developed at other sites were excluded. Studies including cases with a previous history of oral cancer or previous treatment for oral cancer were also excluded.

Data extraction and analysis

Once the final selection of articles for inclusion had been agreed, two researchers (JS, TR) independently extracted data using a standardised data table (Table 1) and a third researcher (HM) checked the data. Two researchers independently assessed the quality of identified studies using the validated, Newcastle Ottawa Scale (NOS), for quality assessment in observational studies.[8] This tool was used to assess the selection method, comparability of cases and exposure. It generates a rating out of nine.

Data on the same marker were grouped. Dichotomous variables were analysed in two by two tables. SAS version 9.1 was used to calculate relative risk, p values and to assess heterogeneity.

Outcome measures

We examined the risk of progression from oral dysplasia to cancer in the presence or absence of identified markers.

Results

Description of studies

Search results

The selection and exclusion process based on the literature search is shown in Fig. 1. Our search strategy identified 2550 citations that were reviewed for suitability for inclusion. 288 citations were identified as warranting further examination. The abstracts

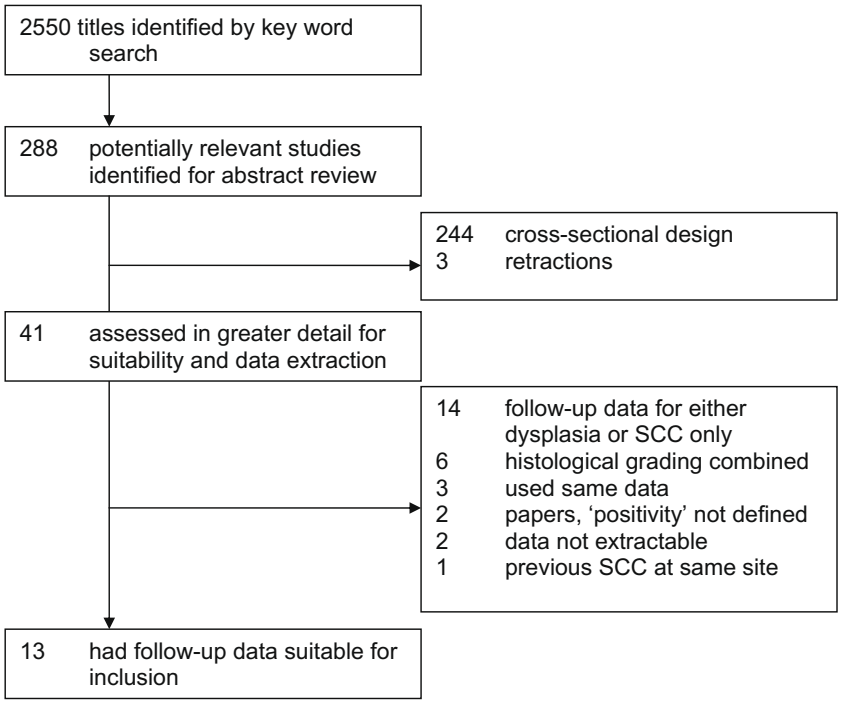

Figure 1 Flow diagram for study selection.

Table 1
Details of selected articles.

Bibliographic citation	Study type	Number of patients	Cases/setting	Marker	Follow-up	Effect size
Cruz et al. (1998)[43]	Retrospective case-control pathology slides	11 Progressing oral lesions. 24 Non-progressing	Samples chosen due to completeness of follow-up. Single centre study. Netherlands	Supra-basal p53	1–16 years. Mean = 5	Presence of suprabasal p53 more specific and better PPV than dysplasia grade. Sensitivity improved when combining with dysplasia grade
General comments: sample chosen due to complete data. No record of case matching for demographic and/or environmental factors. Treatment not recorded						
Hogmo et al. (1998)[45]	Retrospective case-control pathology slides	21 Progressing oral lesions (16 SCC, 5 CIS). 29 Non-progressing. 22 Dysplasia; 11 progressing	Matched for site, grade, age, sex. Single centre study. Sweden	p53, p21, DNA content	Min FU 36 months (mean 112)	No difference in p53 staining for progressing and non-progressing lesions. Nuclear DNA content higher in progressing lesions. p21 Data expressed as %
General comments: single centre. Good case-control matching for site and grade. Small numbers						
Cruz et al. (2006)[44]	Retrospective case-control pathology slides	18 Progressing oral lesions. 18 Non-progressing	Cases selected for complete data on follow-up between 1975 and 1994. Matched controls. Single centre study *N.* Ireland	Supra-basal p53	0.5–16 Years. Mean = 5	No significant difference between p53 and dysplasia grade Low sens (33%), spec (83%) PPV (67%) NPV (56%)
General comments: data for lesions progressing over time not complete. Retrospective data collection. Small numbers of cases						
Rich et al. (1999)[40]	Retrospective cohort pathology slides	30 Cases of dysplasia. 3 Progressed	Single centre: Australia	p53	21–80 Months	Numbers too small to reach significant conclusions. p53 Did not predict outcome
General comments: no comment on case selection. No apparent matching of cases. Only three lesions transformed to SCC during follow-up						
Regezi et al. (1995)[42]	Retrospective cohort pathology slides	19 Cases with sequential biopsies. 10 Progressed	Dual centre: University of California/Hebrew University Jerusalem	p53	8–156 Months	p53 Noted in all grades of dysplasia. p53 Did not predict progression or outcome
General comments: no comment on case selection. Unclear whether consecutive. Possible selection bias. Clinical factors not recorded						
Shahnavaz et al. (2000)[41]	Retrospective case series	8 Cases of dysplasia progressing to SCC	Uncertain cases selection. Single centre	p53	12–92 Months	Six initially positive for wild type p53. No controls for comparison
General comments: prior treatment not recorded e.g. biopsy type						
Chen et al. (2004)[46]	Retrospective cohort. pathology slides	50 Oral dysplasias	Single centre study. Taiwan	p73	Maximum follow-up 2 years	Only 8 cases of SCC available for statistical analyses. No significant prognostic trend demonstrated
General comments: unclear how cases were selected. Cases followed for two years only. Small number of cases progressed						
Lo Muzio et al. (2003)[49]	Retrospective case-control pathology slides	16 Progressing oral lesions. 30 Non-progressing	Single centre. Italy pathology archives 1987–1999	Survivin	13–293 Months	Significant difference between groups. (33% non-progressing vs. 94% progressing)
General comments: retrospective sampling. Uncertain case matching						
Jordan et al. (2004)[47]	Retrospective case-control pathology slides	19 Progressing oral lesions. 15 Non-progressing	Random selection of cases. Single centre. California	MMP 1, 2, 9	Minimum 4 years	No link to dysplasia grade Significant differences between progressors and non-progressors in spite of small numbers MMP1 $p = 0.04$, MMP9 $p = 0.002$ (OR not presented)
General comments: cases selected randomly from large database and attempts made to match cases. Duration of follow-up						
Macabeo-Ong et al. (2003)[48]	Retrospective case-control pathology slides	16 Progressing lesions. 17 Non-progressing	Random selection of cases. Single centre. California	Cathepsin L mRNA	Minimum follow-up 2 years	No link between dysplasia and level of expression. No statistical difference between groups
General comments: random selection of cases. Limited follow-up of non-progressing lesions. Insufficient sample size. No significant result based on this sample						
Partridge et al. (1998)[50]	Prospective cohort study. Pathology slides	16 Progressing, 15 Non-progressing	Cases initially biopsied and then followed every 6/12. Re-biopsies on clinical grounds. Single centre study London	AI (3p, 9p).	24–86 Months mean 60 months	Significant difference in risk of progression to SCC. AI > 2 loci vs. <2 $p = 0.008$
General comments: unclear in method if cases selected and followed prospectively over six monthly periods or identified retrospectively. Possible bias measuring SCC from other sites						
Rosin et al. (2000)[51]	Retrospective case-control pathology slides	54 Non-progressing lesions. 23 Progressing lesions	Single centre. Canada	LOH (3p, 9p)	Average FU 37 months progressing, 96 months non-prog.	Strongest correlations for 3p, and 9p alone and in combination. RR increases with LOH at other sites
General comments: data collected retrospectively. Centralised biopsy centre. Cases and controls matched						
Zhou et al. (2005)[52]	Retrospective case-control pathology slides	14 Progressing lesions 13 Non-progressing lesions	USCF Oral Pathology Tissue Bank. Single centre California	LOH 8p, 11p	6 Years (mean 10.3)	Trends observed no significant differences reported
General comments: small sample size. No comment on case matching or random selection						

and full papers were assessed to determine study methodology. 244 studies employed cross-sectional design with 41 studies containing follow-up data. Three follow-up studies had been retracted from the literature since being published.[9–11] Overall data on

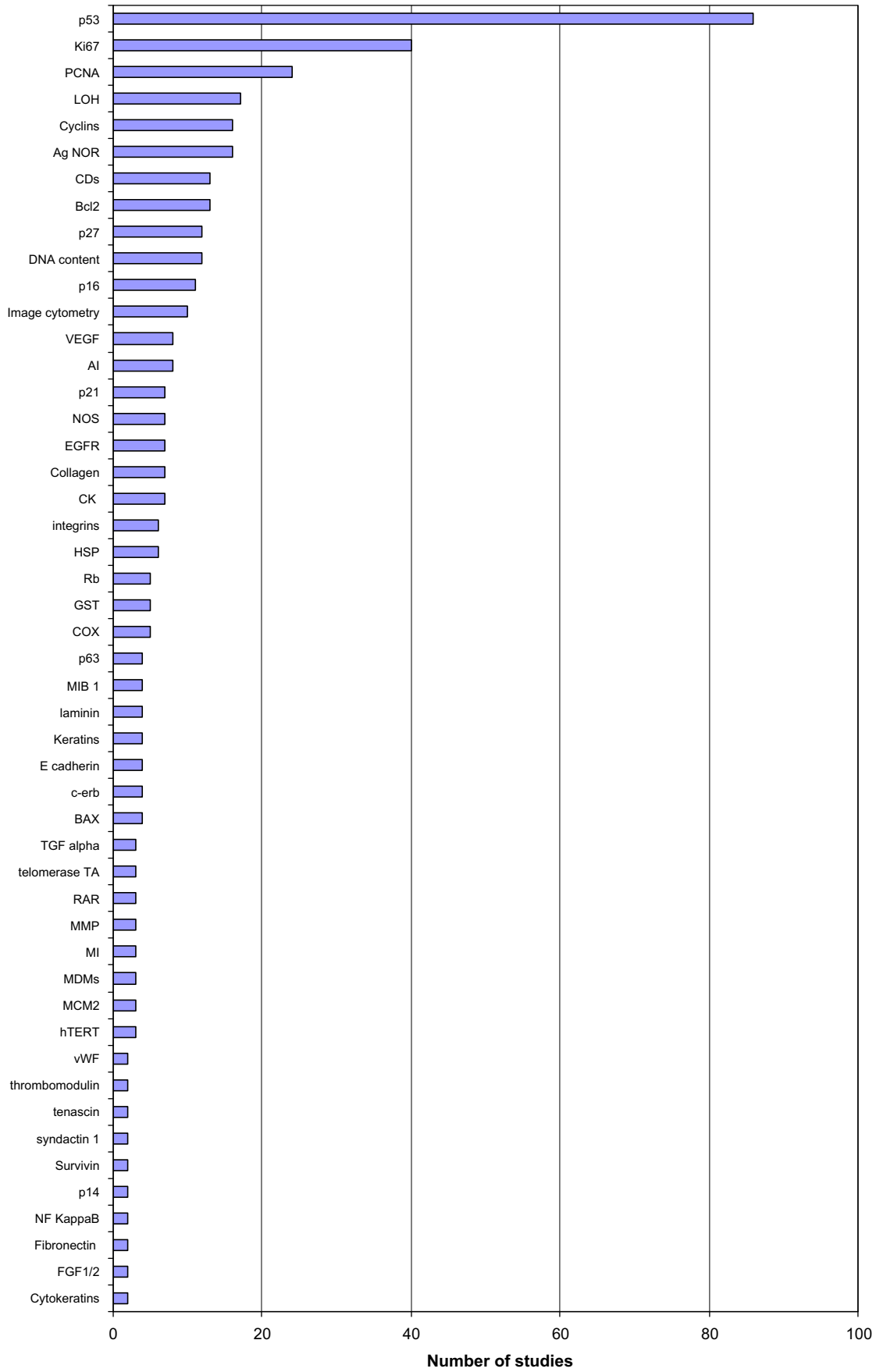

Figure 2 Frequency of studied markers identified by the search.

marker type and frequency from both longitudinal and cross-sectional studies are shown in Fig. 2.

Only 41 studies were longitudinal in design and potentially eligible for inclusion. In 14 of these studies follow-up data were not complete. Cases were either only followed once cancer had developed or consisted of only dysplastic lesions that did not progress. These studies were excluded.[12–25]

In six studies, histological definitions were not used in case selection. Most commonly, hyperplastic and dysplastic oral lesions were combined and presented together. Studies were excluded when the data could not be separated in published tables.[26–31] In a further two studies, a clear definition of marker positive or marker negative cases was not given.[32,33] Markers were expressed as percentages or proportions rather than as positive of negative and as such could not be included in dichotomous analyses.

In three studies, there was duplication of data. One of each was excluded.[34–36] In two studies, specific data on oral dysplasia could not be extracted from the published results tables.[37,38] In the final excluded study, there was a past history of oral cancer at the studied site.[39] For both included and excluded studies, previous treatment and biopsy type was poorly reported.

Included studies

A total of 389 cases from thirteen studies[40–52] were included in this review (Table 1). Of these, 186 dysplastic lesions progressed to cancer and 203 did not. Seven biomarkers (p53, p73, cathepsin L mRNA, matrix metalloproteinase mRNA (MMP1, 2, 9), survivin) and three genetic abnormalities (loss of heterozygosity (LOH), allelic imbalance (AI) and DNA content) were studied longitudinally. Two studies were dual-centre and the remaining were single centre. The number of cases per study ranged from 8 to 50 with a mean of 30 cases. Follow-up ranged between 0.6 and 16 years with a mean follow-up of 3 years for lesions that progressed and 7 years for non-progressing comparisons. The quality of individual studies as defined by the NOS ranged between three and seven out of nine with a median of six. See Table 2.

A total of 113 markers were identified. The graph below show the number of studies identified for each marker. The graph shows result for markers studied in two or more studies only.

Outcomes

For cohorts and case series, odds ratios (OR) were used as an estimate of the relative risk (RR). A correction of 0.5 was used in cells containing zero. Table 3 shows relative risk and confidence intervals (95% CI) for identified markers. Where pooled relative risks are quoted they are fixed effects estimates.

Biomarkers and progression to cancer

p53. We identified six longitudinal studies of p53 with one cohort[40] two case series[41,42] and three case-controls.[43–45] Studies measured p53 staining in both basal and suprabasal layers. A funnel plot to assess the potential influence of publication bias was of limited use due to the number of studies available. The pooled relative risk for cancer progression in p53 positive cases was 0.96 (0.65, 1.42). For p53 staining in suprabasal layers only the relative risk was 1.36 (0.27, 6.82). The p values for heterogeneity were 0.38 and 0.94, respectively. Insufficient data were available to perform sensitivity analysis.

p73, Matrix metalloproteinase mRNA (MMP), cathepsin L mRNA and survivin. We identified one study for each of the above markers.[46–49] The p73 study was a cohort, with the other studies being case-control by design. The relative risk of cancer progression in p73 positive cases was 6.44 (0.71, 84.05). Relative risk of cancer progression in MMP 1 and 2 positive cases was 2.50 (0.58, 10.71) and 2.91 (0.65, 12.74), respectively. Relative risk for progression of MMP 9 positive cases to cancer was 19.00 (1.56, 209.38) $p = 0.02$. For cathepsin L mRNA the relative risk of progression to cancer was 2.50 (0.61, 10.31). The relative risk of progression to cancer in survivin positive cases was 30 (4.25, 197.73), $p \leqslant 0.001$.

Genetic abnormalities and progression to cancer

Loss of heterozygosity (LOH), allelic instability (AI) and DNA content. We identified three studies; one cohort[50] and two case-control,[51,52] studying LOH at multiple loci or allelic instability. Data from the two case-control studies suggest increased risk for LOH at 3p, 8q, 9p and 11p. The greatest effect size was observed at 3p ± 9p, with a relative risk of progression to cancer of 17.60 (2.77–108.37) $p < 0.001$. p Values for heterogeneity at 8p, 9p, and 11q were 0.002, 0.35 and 0.14, respectively. The relative risk of progression in lesions with AI > 2 loci was 3.20 (1.49, 6.33) $p = 0.004$. One case-control study of DNA content compared diploid and non-diploid lesions.[45] Relative risk for progression to cancer of non-diploid cases was 12.00 (1.17, 82.10) $p = 0.03$.

Discussion

Currently, there is not a substantial body of strong evidence for the use of biomarkers in the prognosis of oral dysplasia. There is a suggestion from the longitudinal studies included in this review that the presence of LOH/AI at specific loci, survivin and MMP 9 positivity, and DNA content (non-diploid) increase the risk of progression. Their usefulness in the clinical setting however needs further evaluation. No increased risk of progression of cases positive

Table 2
Quality assessment of included studies using Newcastle Ottawa Scale (NOS).

Study author	Design	Marker	NOS quality assessment			
			Selection (4)	Comparibility (2)	Exposure (3)	Total
Rich et al.[40]	Cohort	p53	*	*	*	3/9
Cruz et al.[44]	Case-control	p53	***	*	**	6/9
Cruz et al.[43]	Case-control	p53	*	*	**	4/9
Shahnavaz et al.[41]	Case-series	p53	*	*	*	3/9
Regezi et al.[42]	Cohort	p53	**	*	**	5/9
Hogmo et al.[45]	Case-control	p53 DNA content	*	**	**	5/9
Jordan et al.[47]	Case-control	MMP 1, 2 + 9	**	*	***	6/9
Macabeo-Ong et al.[48]	Case-control	Cathespin L mRNA	**	*	***	6/9
Partridge et al.[50]	Cohort	Allelic instability	**	*	***	6/9
Rosin et al.[51]	Case-control	LOH	***	**	**	7/9
Zhou et al.[52]	Case-control	LOH	***	*	**	6/9
Lo Muzio et al.[49]	Case-control	Survivin	***	*	***	7/9
Chen et al.[46]	Cohort	p73	***	*	*	5/9

Table 3
Risk of malignant progression in oral dysplasia.

Marker	Study	RR	95% CI	*p* value
p53	Rich et al.[40]	1.04	(0.06, 17.45)	
	Cruz et al.[44]	1.25	(0.09, 17.98)	
	Cruz et al.[43]	1.43	(0.18, 11.09)	
	Shahnavaz et al.[41]	1.09	(0.68, 1.74)	
	Regezi et al.[42]	0.48	(0.20, 1.87)	
	Hogmo et al.[45]	2.86	(0.24, 33.90)	
	Pooled results			
	p53	0.96	(0.65, 1.42)	0.84
	Suprabasal p53	1.36	(0.27, 6.82)	0.71
LOH (3p ± 9p)	Rosin et al.[51]	17.60	(2.77, 108.37)	<0.001
(3p)	Rosin et al.[51]	5.39	(1.88–15.45)	0.003
(9p)	Rosin et al.[51]			
	Zhou et al.[52]			
	Pooled results	3.92	(1.50, 10.25)	0.006
(8p)	Rosin et al.[51]			
	Zhou et al.[52]			
	Pooled results	2.29	(0.87, 6.02)	0.11
(11q)	Rosin et al.[51]			
	Zhou et al.[52]			
	Pooled results	2.86	(1.11, 7.39)	0.02
AI > 2	Partridge et al.[50]	3.20	(1.49, 6.33)	0.004
Cathespin L mRNA	Macabeo-Ong et al.[48]	2.50	(0.61, 10.31)	0.29
Survivin	Lo Muzio et al.[49]	30.00	(4.25, 197.73)	<0.001
MMP 1 mRNA	Jordan et al.[47]	2.50	(0.58, 10.71)	0.28
MMP 2 mRNA	Jordan et al.[47]	2.91	(0.65, 12.74)	0.27
MMP 9 mRNA	Jordan et al.[47]	19.00	(1.56, 209.38)	0.02
p73	Chen et al.[46]	6.44	(0.71, 84.05)	0.28
DNA content	Hogmo et al.[45]	12.00	(1.17, 82.10)	0.03

for p53, p73, cathepsin L mRNA and MMP 1 and 2 was demonstrated.

Limitation of the review

Definitions and study design

In spite of a large number of studies identified by this review, the presence of quality follow-up data for oral dysplasia is limited. The majority of the studies examining markers are cross-sectional analyses, do not provide data on oral lesions over time and are therefore unsuitable to use in analysis of prognosis. Furthermore, there is inconsistency in the literature regarding assignment of marker positive and marker negative cases making studies of the same marker more difficult to compare. Of the few studies in which follow-up data were recorded, issues of definition of cases (clinical vs. pathological) reduce the ability to draw stronger conclusions.

Of the studies included in this review, retrospective data collection and convenience sampling are significant potential sources of bias. Analysis of the effect of interventions, including environmental risk factors, was rarely addressed and has the potential to lead to bias in unmatched studies. Analysis of progression by dysplasia grade was often not published and as such could not be further analysed in this review. The method of biopsy (incisional vs. excisional) of dysplastic lesions is rarely recorded and could lead to treatment bias through the tendency to excise higher grade lesions more completely. Furthermore the small sample sizes may mask the presence of significant results for some of the markers that were shown to have no predictive value.

Quality of evidence

In terms of relative risk for cancer progression, the conclusions reached by this review are based mainly on the results of retrospective, single centre, observational studies in which there are significant potential sources of bias. Furthermore, the studies identified were generally small with an average of 30 cases per study. The numbers of cases eligible for analysis were further restricted by the way in which data had been published.

Other potential biases

We also acknowledge the potential presence of publication bias within this review. With small numbers of studies included, more detailed assessment of the likely effect size could not be performed. No unpublished data were included and further bias may be introduced by limiting the review to only studies published in the English language. Furthermore, data used for pooled analysis of relative risk were taken from published articles rather than original data, potentially limiting its quality.

Implications for research

In spite of a significant body of work in the field of oral dysplasia, it has been directed towards mechanistic exploration rather than clinical application. We identified well over 200 papers in which cross-sectional methods were used. These serve only as snap shots in time and do not contribute to the understanding of the behaviour or natural history of these lesions. Clinical application of these findings is therefore limited. The preponderance of this type of study perhaps highlights the difficulty in accessing complete follow-up data, and to the small number of cases treated in individual centres. We would suggest that collaboration in this field, with multi-centre studies and patients stratified by treatment type, is necessary to further address the issues outlined in this review.

We recommend that in studies of oral lesions, clear distinctions be made between histological and clinical definitions. In those studies where both leukoplakia and dysplasia are studied, we recommended that data are clearly presented separately such that progression can be more accurately linked to the presenting lesion. Where markers are studied in precancerous lesions, a clear and agreed definition of positive and negative cases is also needed in order to stratify risk appropriately.

Implications for practice

The potential has been demonstrated for the use of some markers in stratifying cancer risk in patients with oral dysplasia. If these findings are reproducible and reliable they have potential to change clinical practice in terms of targeting of treatment and follow-up. It is clear that further clinical work is needed in this field and we recommend multi-centre collaboration as the best model for this.

Conflict of Interest Statement

None declared.

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