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Short Communication

Relationship of DNA aneuploidy with distinctive features of oral potentially malignant disorders: A cytological analysis of 748 cases

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Abstract Our previous study reported that clinical features, including the lateral/ventral tongue and non-homogeneous lesions, were associated with increased risk of malignant changes in cytological samples from oral potentially malignant disorders (OPMDs). This cross-sectional study aimed to evaluate the frequency and risk of DNA aneuploidy in the series of 748 patients with OPMD. The cut-off value of aneuploidy was defined as DNA index $\geq$3.5. We found that the frequency of DNA aneuploidy was higher in OPMD patients >60 years old, and in those with lateral/ventral tongue sites, non-homogeneous lesions, and high-grade dysplasia, than in control group ($P < 0.01$). Consistently, the risk of aneuploidy occurrence was higher in patients >60 years old (1.69-fold; $P = 0.022$), in those with lateral/ventral tongue sites (2.35-fold; $P < 0.001$), and in those with high-grade dysplasia (3.19-fold; $P < 0.001$).

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Collectively, increased frequency and risk of DNA aneuploidy occurred in OPMD patients aged over 60 years with high-grade dysplasia located at the lateral/ventral tongue. These patients should be required to intensive management and follow-up.

Introduction

Oral potentially malignant disorders (OPMDs) carry a significantly increased risk of progression to oral squamous cell carcinomas (OSCCs).[1,2] Tobacco smoking, high-risk site at tongue/mouth floor, and oral dysplasia are known risk factors of OSCC development.[1,2] Image cytometry using oral brushings is an objective and noninvasive adjunctive diagnostic technique to automatically measure nuclear DNA content and determine DNA ploidy status.[3] In a large prospective series of OPMDs, we previously reported that the lateral/ventral tongue site, non-homogeneous lesions, and tobacco smoking are significant factors associated with increased risk of malignant changes.[4] Furthermore, we established a risk model based on DNA aneuploidy that consisted of a noninvasive strategy with lateral/ventral tongue and non-homogeneous features.[5]

Currently, the relationship between DNA aneuploidy and the clinicopathological risk factors of OPMDs, including smoking, patient age, lesion site, and dysplasia, are unclear.[6] To date, reports in the literature should be considered preliminary due to the small sample sizes and different methods (image cytometry or flow cytometry) used to measure aneuploidy.[7] In this context, we hypothesized that DNA aneuploidy was significantly associated with distinctive risk factors of general OPMD. Hence, this cross-sectional study aimed to analyze whether higher frequency and risk of aneuploidy with DNA-image cytometry (DNA-ICM) was associated with OPMD patients presenting with these risk factors.

Materials and methods

The clinical and histological diagnoses of the enrolled OPMD patients were based on the definition and criteria described previously.[4,5] Our series enrolled 810 patients, including 748 cases of general OPMDs and 62 cases of OPMD concomitant OSCCs.[4,5] The current study focused on the 748 cases of general OPMDs and excluded the 62 cases of OPMD concomitant OSCCs. As our described previously,[4,5] oral brushing (Oral cytobrush kits; CytoSavant, Motic Inc., Xiamen, China) and biopsy samples taken from each subject were processed by DNA image cytometer (MotiSavant, Motic Inc., Xiamen, China) and histopathological examination, respectively. Based on the optimization criteria reported in our previous study,[5,6] at least one aneuploid cell with DNA index (DI) $\geq$3.5 was defined as the cut-off value of aneuploidy in this study. This study was approved by the Institutional Review Board of Shanghai Ninth People's Hospital (SH9H-[2012]21) and written informed consent was obtained from the subjects.

Statistical analysis was performed using SPSS for Windows (version 21.0; SPSS Inc.). Differences in qualitative variables were calculated by the chi-square test or Fisher's exact test. Binary logistic regression analysis was applied to evaluate the odds ratio (OR) with 95% confidence interval (CI) and the association among dichotomous variables. In logistic regression, univariate analysis was first performed to obtain the significant variables. To further assess and adjust the influence of each significant variable, multivariate analysis was then performed to assess which factors remained statistically significant. All tests were two-sided, and P-values of $<$0.05 were considered statistically significant.

Results

According to the criteria of at least one aneuploid cell with DI $\geq$ 3.5,[5] 120 of 748 (16.0%) OPMD patients were identified as DNA aneuploidy. The distribution of normal (DI $<$ 3.5)

Table 1 Distribution of normal (DNA index (DI) $<$ 3.5) and abnormal (DI $\geq$ 3.5) DNA content among 748 patients with oral potentially malignant disorder by clinicopathological features.

Characteristic	DI $<$ 3.5	DI $\geq$ 3.5	χ2 test
Total, n (%)	628 (84.0)	120 (16.0)	P value
Age group (years)			$<$0.001
$\leq$ 60	459 (87.1)	68 (12.9)	
$>$ 60	169 (76.5)	52 (23.5)	
Gender			0.320
Female	313 (82.6)	66 (17.4)	
Male	315 (85.4)	54 (14.6)	
Tobacco smoking			0.239
No	376 (82.6)	79 (17.4)	
Yes	211 (86.1)	34 (13.9)	
Unkonwn	41	7	
Alcohol drinking			0.659
No	404 (84.3)	75 (15.7)	
Yes	183 (82.8)	38 (17.2)	
Unkonwn	41	7	
Lesion site			$<$0.001
Others	502 (88.4)	66 (11.6)	
Lateral/ventral tongue	126 (70.0)	54 (30.0)	
Lesion type			0.005
Homogeneous	476 (86.2)	76 (13.8)	
Non-homogeneous	152 (77.6)	44 (22.4)	
Degree of dysplasia			$<$0.001
Non/mild	544 (88.5)	71 (11.5)	
Moderate/severe	84 (63.2)	49 (36.8)	

and abnormal (DI ≥ 3.5) DNA content among OPMD patients by clinicopathological features are presented in Table 1. The frequency of DNA aneuploidy in patients >60 years old (23.5%) was significantly higher than that in patients ≤60 years old (12.9%) (P < 0.001). The frequency of DNA aneuploidy in lateral/ventral tongue sites (30.0%) and non-homogeneous lesions (22.4%) was significantly higher than that observed in other sites (11.6%) and homogeneous lesions (13.8%) (both P < 0.01). The frequency of DNA aneuploidy in moderate/severe (high-grade) dysplasia (36.8%) was higher than that in non-/mild dysplasia (11.5%) (P < 0.001). Representative clinical manifestation, DNA content analysis, and histopathology of two cases of OPMDs are shown in Supplementary Fig. S1.

To further assess the association between the occurrence of DNA aneuploidy and the clinicopathological features of OPMDs, risk factors were analyzed using the logistic regression model (Table 2). Univariate analysis revealed that patients >60 years old, lateral/ventral tongue sites, non-homogeneous lesions, and high-grade dysplasia were significantly associated with a higher risk of DNA aneuploidy occurrence. Multivariate analysis revealed that patients >60 years old (P = 0.022), and those with lateral/ventral tongue sites (P < 0.001) and high-grade dysplasia (P < 0.001), had significantly increased risk of DNA aneuploidy occurrence. Conversely, the risk occurrence of DNA aneuploidy in tobacco smokers was 0.52-fold (95% CI, 0.28–0.94; P = 0.040) less than in non-smokers.

Discussion

DNA aneuploidy is an indicator of numerical chromosomal changes and its occurrence is typically an early crucial step in carcinogenesis.[3] In previous years, a research team from Italy reported a series of studies on the application of DNA-flow cytometry in the adjunctive diagnosis of OPMDs/OSCCs.[8–10] In recent years, we have focused on examining the application of DNA-ICM in the adjunctive diagnosis of OPMDs.[4–6] In the current study, we found that patient age was a risk factor affecting DNA aneuploidy, consistent with the Italian study.[9] It was further reported that the aneuploid frequency of oral dysplasia and tongue lesions was higher than that of oral non-dysplasia and buccal lesions, respectively.[9,10] We also found that aneuploid frequency and risk of moderate/severe dysplasia and lateral/ventral tongue lesions was higher than non-/mild dysplasia and other oral sites, respectively. Furthermore, we found that the aneuploid frequency of non-smokers with OPMDs was slightly higher than that of smokers, in contrast to previous studies.[8] Moreover, we showed that non-homogeneous lesions were a risk factor of OPMD patients and affected DNA aneuploidy. Non-homogeneous lesions, moderate/severe dysplasia, lateral/ventral tongue sites, and elderly patients have previously been shown to be risk factors of OPMD malignant progression.[4] Further studies are required to investigate the association of DNA aneuploidy with clinicopathological factors of OPMD.

Table 2 Risk assessmentof DNA aneuploidy (DNA index ≥3.5) in 748 patients with oral potentially malignant disorder.

Characteristic	Univariate analysis		Multivariate analysis	
Total, n (%)	OR (95%CI)	P value	OR (95%CI)	P value
Age group (years)				
≤60	1.0 (ref)		1.0 (Ref)	
>60	2.08 (1.39–3.10)	<0.001	1.69 (1.08–2.64)	0.022
Gender				
Female	1.0 (ref)		1.0 (ref)	
Male	0.81 (0.55–1.20)	0.301	1.27 (0.74–2.17)	0.387
Tobacco smoking				
No	1.0 (ref)		1.0 (ref)	
Yes	0.77 (0.50–1.19)	0.233	0.52 (0.28–0.97)	0.040
Unkonwn				
Alcohol drinking				
No	1.0 (ref)		1.0 (ref)	
Yes	1.12 (0.73–1.72)	0.608	1.81 (0.99–3.31)	0.053
Unkonwn				
Lesion site				
Others	1.0 (ref)		1.0 (ref)	
Lateral/ventral tongue	3.26 (2.17–4.91)	<0.001	2.35 (1.47–3.78)	<0.001
Lesion type				
Homogeneous	1.0 (ref)		1.0 (ref)	
Non-homogeneous	1.81 (1.20–2.74)	0.005	0.97 (0.59–1.60)	0.916
Degree of dysplasia				
Non/mild	1.0 (ref)		1.0 (ref)	
Moderate/severe	4.47 (2.91–6.87)	<0.001	3.19 (1.91–5.35)	<0.001

Abbreviations: CI, confidence interval; OR, odds ratio.
The "(ref)" means that one variate (e.g. male) of the dichotomous variates refers the other one (e.g. female) in the analysis.

The inconsistent classification criteria of DNA aneuploidy is the most one of limitations for diagnostic evaluation in cytological samples from OPMD/OSCC patients.[7] In the majority of the previous studies, outside DI of 1.8—2.2 and 3.6—4.4 and/or 9c events was defined as aneuploidy criteria; whilst cells with DI $\geq$ 2.3 was also defined as DNA aneuploidy criteria used by some other studies (summarized in ref. 7). It is noteworthy that the various proportions of carcinoma and dysplasia enrolled in a study could produce different results, since detection of carcinoma and dysplasia are the exposed factors. Moreover, both OPMD and OSCC patients were enrolled in the previous studies,[7] and there was lack of the reports on DNA aneuploidy in patients with clinical diagnosis of OPMD alone. Consequently, we recently conducted a large-scale series (n = 401) in a single study on the diagnostic value of the DNA-ICM to optimize cut-off values of DNA content for noninvasive detection of oral carcinoma and dysplasia within OPMDs with homogeneity of the enrolled subjects.[6] The study showed that the optimal cut-off of aneuploidy for detecting dysplasia within OPMDs was cells with DI $\geq$ 2.3,[6] in agreement with the criteria defined by Parfenova et al.[11] and Datta et al.[12] Meanwhile, the optimal cut-off of aneuploidy for detecting malignant changes within OPMDs was cells with DI $\geq$ 3.5,[6] which was further validated by a larger-scale series (n = 810) in our subsequent study.[5] In the current report, we utilize the same series (n = 810) of OPMDs and excluded the 62 cases of OPMD concomitant OSCCs. We only focus on the 748 cases of general OPMDs to analyze the relationship of DNA aneuploidy with distinctive features of patients with general OPMDs. According to the same criteria (DI $\geq$ 3.5) for detecting malignant changes within OPMDs,[5] we analyze the risk factors of patients with DI $\geq$ 3.5 versus those with DI $<$ 3.5. We are aware of the limitations of our study including the cross-sectional study setting and lack of clinical endpoint data of malignant transformation. Longitudinal studies on DNA-ICM using oral brushing samples collected at different time points during follow-up, especially in patients with DI $\geq$ 3.5, as a surveillance tool for oral cancer progression are warranted. It is mandatory for patients with DI $\geq$ 3.5 to receive more careful surveillance and aggressive treatment, so as to detect early malignant changes.

Collectively, this large-scale cross-sectional study evaluated the frequency and risk of DNA aneuploidy in OPMD patients with significant risk factors including age over 60 years, lateral/ventral tongue sites, non-homogeneous lesions, and high-grade dysplasia. OPMD patients presenting with these risk factors had a higher frequency and risk of DNA aneuploidy than controls. High-risk patients associated with DNA aneuploidy deserve closer clinical follow-up and earlier therapeutic interventions on the basis of a higher potential of malignant transformation. Such features may be beneficial in assisting the clinician to establish appropriate therapies and follow-up schedules.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2021.10.010.

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