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Micronucleus frequencies in lymphocytes and buccal epithelial cells from patients having head and neck cancer and their first-degree relatives

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Head and neck squamous cell carcinoma is the fifth most common cancer type worldwide. Even though it is known that the most important environmental aetiological factors for head and neck cancer (HNC) development are tobacco and alcohol, genetic susceptibility is also thought to be important. The use of biomarkers of chromosomal damage due to genetic instability in order to predict risk of cancer as well as to identify high-risk individuals is imperative. We have investigated genetic damage in patients having HNC (n = 59) and their first-degree relatives (FDRs) (n = 34) with a biomarker in two different tissues; the micronucleus (MN) test in peripheral blood lymphocytes and in exfoliated buccal cells. The mean (standard deviation) levels of MN frequencies (‰) in lymphocytes of patients, relatives and controls were 27.10 (9.52), 14.09 (5.13) and 9.00 (6.87), respectively. The mean (standard deviation) levels of MN frequencies (‰) in exfoliated buccal cells of patients, relatives and controls were 2.87 (1.16), 1.38 (0.85) and 1.23 (0.93), respectively. Our results indicated that spontaneous genetic damage in lymphocytes of patients having HNC was significantly higher than that of controls (P < 0.01) and thus genetic instability appeared to exist in lymphocytes of cancer patients. Similar findings were obtained for exfoliated buccal cell MN frequencies of cancer patients (P < 0.01). We observed that the FDRs of patients having HNC showed significantly higher chromosomal damage in terms of MN frequencies in lymphocytes when compared with those of controls (P < 0.05), thus reflecting an increased susceptibility to HNC in FDRs. However, for buccal cell MN frequencies, we could not demonstrate enhanced genetic instability in the FDRs of patients having HNC.

Introduction

Head and neck squamous cell carcinoma is the fifth most common cancer type worldwide; however, there are considerable differences in incidence and it is more common in developing regions, with >500 000 new cases each year (1,2).

The development of head and neck cancer (HNC) depends on both environmental and genetic factors (3,4). Even though it is known that the most important environmental aetiological factors for HNC development are tobacco and alcohol, genetic susceptibility is also thought to be crucial (1,2). Familial aggregation may be an indicator of inherited susceptibility. Heritable familial susceptibility for HNC has been supported by family studies indicating increased risk for the first-degree relatives (FDRs) of patients having HNC (5–8).

Genetic instability may affect pathways such as enzymes that replicate and repair DNA, proteins that affect chromosomal stability, proteins that control apoptosis and cell cycle regulation in response to DNA damage. Mutations in each of these pathways may have been related to cancer development (9). Since the latency periods in most cancer types are usually 15–20 years or longer, conducting prospective epidemiological studies over long periods is not always practical. Therefore, it is imperative to use some biomarkers of DNA damage due to genetic instability to predict cancer risk as well as to identify high-risk individuals (10). Cytogenetic biomarkers are the most frequently used end points in human population studies. One of the cytogenetic biomarkers for predicting cancer risk in humans is the micronucleus (MN) test in peripheral blood lymphocytes (PBL) (11,12).

As well as the MN test in PBL, the MN test in exfoliated buccal cells is an attractive candidate for the genotoxic biomonitoring of human populations and individuals, especially because of its non-invasive application nature. It is considered to be a useful biomarker of genetic damage caused by lifestyle habits, exposure to environmental pollutants, medical procedures and also inherited genetic defects in DNA repair (13–18). Rajeswari *et al*. (19) reported that significant increases in MN frequency in exfoliated buccal epithelial cells were found in both breast cancer patients and their FDRs when compared to healthy subjects. It is important to identify the individuals who are more susceptible to cancer in HNC families by using the early predictors of genetic damage. To date, no single biomarker has been available which could be used to identify individuals at high risk in FDRs of patients having HNC. In our study, we have investigated the genetic damage in patients having HNC and their FDRs with a biomarker in two different tissues; the MN test in PBL and in exfoliated buccal cells.

Materials and methods

Subjects

Venous blood samples were obtained from 59 patients having HNC that were confirmed pathologically, 34 healthy FDRs (as 6 parents, 12 siblings or 16 sons/daughters) of patients having HNC and 31 healthy control volunteers who did not have any HNC history in their family. There was one 8-year-old child having nasopharyngeal cancer in the patients group and one 8-year-old child in the FDR group. Cancer cases included 32 patients with squamous carcinoma of the larynx, 14 of the nasopharynx, 10 of the oral cavity and 3 of the paranasal sinuses. None of the subjects had any chemotherapy or radiotherapy except for

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two patients who had chemotherapy >6 months before the sampling. Buccal epithelial cells were collected simultaneously with the peripheral blood samples from patients having HNC and FDRs as well as controls. Each subject completed a detailed questionnaire including age, gender, occupation data, as well as smoking, alcohol drinking and dietary habits family history of cancer and a brief medical history. Almost all of the subjects had mixed diet. Current smokers were defined as those who have been smoking at least 1 year before blood collection. Former smokers were defined as those who had smoked cigarettes but had stopped smoking >1 year prior to blood collection. Alcohol consumption was quantitatively evaluated, based on self-reported data. The main demographic data are summarised in Table I. This study was approved by the local ethical committee of the Ahmet Andiçen Oncology Hospital, Ankara, Turkey. All subjects were informed about the study and gave a written consent for the participation.

Cytochalasin-blocked MN frequencies in lymphocytes

Culture samples consisted of RPMI medium (5 ml; Sigma) supplemented with 20% foetal calf serum (Sigma), 2% phytohemagglutinine (Sigma) and 0.5 ml of whole blood. All experiments were carried out in duplicate. Cell culture samples were incubated at 37°C for 72 h. Binucleated cells were accumulated by adding cytochalasin B (Sigma) to a final concentration of 6 μg/ml at the 44th h following the initiation of the culture sample. At the end of 72 h, samples were centrifuged and re-suspended in 0.075 M KCl at 4°C for 3 min for hypotonic treatment. Cells were fixed with methanol–acetic acid (3:1, v/v), washed three times, spread onto cold slides and air-dried. Slides were stained with May-Grünwald and Giemsa. The induction of MN was evaluated by scoring a total of 1000 binucleated cells per subject at ×1000 magnification. MN were scored according to the criteria described by Fenech (20), only in binuclear lymphocytes in which the nuclei were of equal size and of the same colour. The diameter of the MN was between 1/16 and 1/3 of the main nuclei and there was no link between the two via a nucleoplasmic bridge. MN frequencies were expressed as MN per thousand (‰). The nuclear division index (NDI), a cell proliferation index, was also calculated according to Eastmond and Tucker (21).

MN frequencies in exfoliated buccal cells

Subjects were asked to rinse their mouths with water and a pre-moistened wooden tongue depressor was used to sample cells from both sides of the interior cheek. The spatula was applied to a pre-cleaned and pre-moistened microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Feulgen reactive and then counterstained with Fast Green. Criteria of scoring were described by Sarto *et al*. (22) and Tolbert *et al*. (23). A total of 1500 differentiated and basal cells per subject were scored blindly by the same scorer. Degenerated cells were observed but not scored. MN frequencies were expressed as MN per thousand (‰).

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 11.5. Whether the continuous variables were distributed normally or not, they were determined by using Shapiro Wilk test. Levene test was applied for the evaluation of the homogeneity of variances. Data were shown as mean ± standard deviation or median (minimum–maximum) for continuous variables, where appropriate. While means were compared by Student's *t*-test or one-way analysis of variance (ANOVA), regarding the number of independent groups, in the comparisons of medians on the other hand, the Mann–Whitney *U* or Kruskal–Wallis test was applied, where applicable. When the *P*-value from the one-way ANOVA or Kruskal–Wallis test statistics was statistically significant, post hoc Tukey or nonparametric multiple comparison test was used to determine which groups differ from which others. Nominal data were evaluated by Pearson's chi-square or Fisher's exact test, where applicable. Degrees of association between continuous variables were evaluated by either Pearson's product-moment or Spearman's rank correlation test. Multiple linear regression analyses with adjustment for potential confounding factors were then performed to determine the pure effect of cancer status on MN frequencies in lymphocytes and buccal cells, respectively. Coefficients of regression and 95% confidence intervals for all independent variables were calculated. Logarithmic transformations were applied for all dependent variables in linear regression analyses because the variables were not normally distributed. A P value <0.05 was considered statistically significant.

Table I. Demographic characteristics of study subjects

	Patients	Relatives	Controls
N	59	34	31
Mean age (±SD)	54.1 (±15.4)[a]	41.2 (±15.0)	45.0 (±15.3)
(minimum–maximum)	(8–85)	(8–70)	(21–78)
Gender *N* (%)[b]			
Male	52 (88.1)	26 (76.5)	27 (87.1)
Female	7 (11.9)	8 (23.5)	4 (12.9)
Smoking status *N* (%)[c]			
Never-smokers	15 (25.4)	11 (32.4)	6 (19.4)
Former smokers	13 (22.0)	5 (14.7)	3 (9.7)
Current smokers			
1–10 cigarettes/day	9 (15.3)	4 (11.8)	4 (12.9)
11–20 cigarettes/day	8 (13.6)	8 (23.5)	7 (22.6)
>20 cigarettes/day	14 (23.7)	6 (17.6)	11 (35.5)
Alcohol intake *N* (%)			
Non-drinkers	55 (93.2)	31 (91.2)	22 (71.0)
Drinkers	4 (6.8)	3 (8.8)	9 (29.0)[d]
Alcohol consumption (g/week)[e]			
median (Min.–Max.)	0 (0–560)	0 (0–50)	0 (0–250)

[a]Significantly different from controls and FDRs ($P < 0.05$). [b,c] $P > 0.05$ (chi-square test); [d]Significantly different from patients and FDRs ($P < 0.05$; chi-square test). [e]$P > 0.05$ (one way ANOVA test).

Results

Table I indicates the demographic characteristics of the study subjects. The mean age of the patient group was significantly higher than those of their FDRs and controls ($P < 0.05$). Gender and smoking status were similar among the groups. Alcohol intake was low in the study groups; however, drinkers were more prevalent among control subjects ($P < 0.05$). Alcohol consumption levels were similar in all groups ($P > 0.05$). Proportion of family history of other cancers among FDRs of patients (10.2%) and those of controls (9.7%) were similar ($P > 0.05$).

In Table II, the mean levels of MN frequencies in peripheral lymphocytes are shown for the patients, their FDRs and controls. The mean (standard deviation) levels of MN frequencies (‰) in the patients, relatives and controls were 27.10 (9.52), 14.09 (5.13) and 9.00 (6.87), respectively. MN frequencies in lymphocytes of the patients were significantly higher than those in both relatives and controls ($P < 0.01$; Table II). A statistically significant difference was also found between FDRs of patients having HNC and control subjects ($P < 0.05$; Table II). NDI values of cancer patients and control subjects were similar ($P > 0.05$), whereas those of FDRs of patients having HNC were significantly different from both patients and control subjects ($P < 0.05$ and $P < 0.01$, respectively; Table II).

A total of 151500 buccal epithelial cells were analysed for MN in cancer patients, their FDRs and control subjects. The results of the cytogenetic analysis of buccal smears from cancer patients, their FDRs as well as control subjects are presented in Table III.

A significant increase in MN frequency was found in exfoliated buccal cells of patients when compared with those of

Table II. MN frequencies in peripheral lymphocytes and NDI values of study subject

Group	MN frequency (‰), mean (SD)	(Min.–Max.)	NDI
Patients ($n = 59$)	27.10 (9.52)[a]	12–67	2.01 (0.13)[d]
Relatives ($n = 34$)	14.09 (5.13)[b]	6–28	2.09 (0.13)[e]
Controls ($n = 31$)	9.00 (6.87)[c]	2–31	1.97 (0.16)[f]

[a,c]$P < 0.01$; [a,b]$P < 0.01$; [b,c]$P < 0.05$; [d,f]$P > 0.05$; [e,f]$P < 0.01$; [e,d]$P < 0.05$ (Kruskal–Wallis).

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controls and relatives ($P < 0.01$, Table III). There was a slight increase in MN frequencies in buccal cells from relatives compared to the control group; however, this was not statistically significant ($P > 0.05$, Table III).

There was a significant correlation between MN frequencies in lymphocytes and those in buccal cells for the control group ($r = -0.412$, $P < 0.024$; age adjusted) but not for patients having HNC ($r = 0.104$; $P = 0.512$; age adjusted) and their FDRs ($r = -0.165$; $P = 0.420$; age adjusted). MN frequencies both in lymphocytes and buccal cells by cancer site did not differ significantly ($P > 0.05$).

The modulation of MN frequencies in peripheral lymphocytes and those in buccal cells by several factors was investigated by multiple linear regression analysis (Table IV). Age, gender, cancer status, FDRs of patients having HNC, former smoking, current smoking status and alcohol intake (yes/no) and NDI were considered as independent variables. Regression analysis of data indicated that 60% of the total variance of *ln*MN frequency in lymphocytes could be explained by a highly significant ($P < 0.001$) statistical model where cancer status and their FDRs, and to a lesser extent age, gender and NDI ($P < 0.05$), entered as significant variables, while 23% of total variance of *ln*MN in buccal cells was explained only the cancer status ($P < 0.001$, Table IV).

Table III. MN frequencies in buccal cells of study subjects

Group[a]	MN frequency (‰), mean (SD)	(Min.–Max.)
Patients ($n = 43$)[b]	2.87 (1.16)	(0.67–5.33)
Relatives ($n = 27$)[c]	1.38 (0.85)	(0.00–3.33)
Controls ($n = 31$)[d]	1.23 (0.93)	(0.00–3.33)

[a]Buccal samples from 6 patients and 7 FDRs were not available.
[b,c]$P < 0.01$; [b,d]$P < 0.01$; [c,d]$P > 0.05$ (Kruskal–Wallis).

Table IV. Multiple linear regression analysis of MN data

Variable	Regression coefficient(B)	95% CI	*P*
Dependent variable[a]: ln MN in lymphocytes			
Constant[b]	−0.023	−1.319 to 1.273	0.972
Cancer patients	1.190	0.979–1.401	≤0.001
FDRs	0.548	0.308–0.788	≤0.001
Age	0.008	0.002–0.013	0.005
Gender	0.263	0.021–0.505	0.036
Ex Smoker	0.077	−0.187 to 0.342	0.563
Current Smoker	0.168	−0.040 to 0.376	0.112
Alcohol Intake	–0.105	–0.356 to 0.145	0.406
NDI	0.627	0.025–1.230	0.041
Dependent variable[c]: ln MN in buccal cells			
Constant[b]	−0.379	−1.571 to 0.813	0.529
Cancer patients	1.422	0.901–1.942	≤0.001
First-degree relatives	0.450	−0.116–1.016	0.118
Age	−0.003	−0.016–0.011	0.716
Gender	−0.144	−0.752–0.464	0.639
Ex Smoker	0.381	−0.287–1.048	0.260
Current Smoker	0.229	−0.298–0.756	0.391
Alcohol Intake	0.474	−0.181–1.1129	0.154

[a]Significance of the model: $P < 0.001$, $F = 24.490$, total variance explained adjusted $R^2 = 0.604$. [b]Estimated intercept value.
[c]Significance of the model: ≤0.001, $F = 5.313$, total variance explained adjusted $R^2 = 0.232$.

Discussion

It is known that chromosomal changes and genetic instability are major factors in the carcinogenic process. The search of cytogenetic biomarkers for the identification of groups and/or individuals at high risk of cancer is an important task in public health (24). It has been shown that there is an association between high levels of chromosome aberrations (CAs) and increased risk of cancer in humans (25) and the same association was recently established for MN frequencies in lymphocytes (11,12). MN frequencies represent both clastogenicity (chromosome breakage) and aneuploidy (chromosome loss) in cells studied and it has been extensively used to identify potential genotoxic exposures and also chromosomal instability. The MN assay, when compared with the CA assay, has been relatively easier, faster and did not require metaphase cells (10).

In the present study, the MN assay was used simultaneously to detect baseline genetic damage both in lymphocytes and buccal cells from patients having HNC and their healthy FDRs. Originality of this study comes from the assessment of MN frequencies in two different tissues of FDRs of patients having HNC.

MN frequencies in lymphocytes of patients having HNC and their FDRs

Our results indicated that spontaneous genetic damage in lymphocytes of patients having HNC was higher than that of controls, thus meaning that genetic instability appeared to exist in lymphocytes of patients having HNC. In the present study, greatest chromosomal damage was observed in patients having HNC. This observation is supported by the conclusions of some other studies on patients having HNC (26–28) as well as on patients with different cancers (29).

Duffaud *et al.* (26) suggested that the increase of MN frequencies of only non-smoking patients having HNC may be attributable to cancer status. However, Duffaud *et al.* (26) and Minicucci *et al.* (30) did not demonstrate any significant effect of MN frequencies in smoking patients having HNC, where 84 and 91%, respectively in these studies, were heavy smokers. In our study, the proportion of heavy smokers in patients having HNC and controls was 24 and 36%, respectively. Delfino *et al.* (27) have observed that increasing trend of MN occurred from normal tissue to carcinoma in patients having HNC. Palyvoda *et al.* (28) have also demonstrated that spontaneous chromosomal damage levels as MN frequencies were significantly higher in patients having HNC than in the healthy controls.

Although cigarette smoking and alcohol are the major risk factors for HNC, there is some evidence that genetic susceptibility may also occur (1,2). However, a few studies examined the relationship between HNC risk and family history of cancer (5–8). In the recent pooled analysis by the International Head and Neck Cancer Epidemiology Consortium (31), family history of HNC in FDRs increased the risk of HNC [odds ratio (OR):1.7], especially in siblings (OR:2.2) and more for hypopharynx and larynx sites. The risk was increased (OR:7.2) among subjects with a family history of HNC, who were using tobacco and alcohol.

The importance of genetic familial influence for development of HNC has been shown by using a conventional CA assay in one study (32). Egeli *et al.* (32) observed that CAs of 14 patients having HNC and of 17 healthy FDRs of these patients were significantly higher than those of 20 healthy controls.

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Regarding the MN frequencies in lymphocytes of the FDRs of patients having HNC in the present study, we observed that the FDRs of patients having HNC showed ~1.6 times higher chromosomal damage as compared to controls, thus reflecting an increased susceptibility to HNC in the FDRs. Our observation is consistent with the previous study carried out with CAs as the cytogenetic end point (32). Using the 90th percentile of the lymphocyte MN levels of the control subjects as a cut-off value (16.8‰) for chromosomal instability, 89.8% of the patients having cancer ($n = 53$) and 35.3% of the FDRs ($n = 12$) had higher chromosomal instability compared with 9.7% of the healthy controls ($n = 3$) ($P < 0.01$). Spontaneous MN levels in lymphocytes seem to be a promising indicator of inherited susceptibility in cancer families. This agrees with some findings by Berg-Drewniok *et al*. (33), who demonstrated chromosomal instability as spontaneous MN frequencies in FDRs of familial melanoma patients.

There is sufficient evidence that age, gender, heavy smoking, vitamin B_{12} and folate status may have an impact on the levels of spontaneous MN in lymphocytes (34). In the present study, the study groups were similar in respect to gender and smoking habits distribution, but not to age. By multiple linear regression analysis, HNC status and being the FDRs of patients were the major factors contributing to the significance of spontaneous MN frequencies. However, to some extent, age and gender still had a significant effect on MN frequencies, whereas alcohol and smoking habits did not. These results are consistent with some previous reports concerning the MN assay (10,35).

MN frequencies in buccal cells of patients having HNC and their FDRs

Since exfoliated buccal cells are a good source of tissue for monitoring genetic damage including aneuploidy as well as clastogenicity in humans, they have increasingly been used in many countries (14).

In the present study, the MN test in buccal cells as well as in lymphocytes was used to detect enhanced chromosomal instability in patients having HNC and their FDRs. Our findings revealed that increased chromosomal damage was evident for only patients having HNC. The mean frequency of 2.87‰ MN in buccal cells from our cancer patients was in the range of those of cancer and precancerous patients; 0.60–9.00‰ (14). Regarding the patients having HNC, it was shown that MN percentage was correlated with cancer of the oral cavity (36,37). Bloching *et al*. (38) demonstrated that buccal MN rates were 2-fold higher in pharyngeal cancer patients compared to healthy subjects. Our results are consistent with those of the aforementioned studies. However, Minicucci *et al*. (30) did not show significant MN differences in buccal cells from HNC patients (91% smokers). It is known that several factors such as age, gender, smoking, alcohol intake and dietary habits may contribute to the spontaneous MN levels in exfoliated buccal cells. However, due to some limitations of these studies, the most important confounding factors have not been accomplished yet. These issues have recently been addressed in two review articles (14,39). Ceppi *et al*. (39) estimated buccal MN frequency as 1.10‰ (ranging from 0.70 to 1.72) from a meta-analysis of healthy subjects. Mean MN frequency for our control subjects (1.23‰), fell in that range; however, it must be mentioned that there was a variability (from 0.33 to 1.23‰) in the background levels in buccal cells of healthy subjects from our previous studies [(17,18,40), this study], thus confirming the need of method standardisation by the initiative of the $HUMAN_{xl}$ project (41).

In our study, age, gender, smoking habits and alcohol intake were not confounding factors on buccal MN frequencies. By multiple linear regression analysis, HNC status was the only major factor contributing to the significance of spontaneous buccal MN frequencies. We did not find enhanced genetic instability in buccal cells for the FDRs of patients having HNC. Using the 90th percentile of the buccal MN levels of the control subjects as a cut-off value (2.67‰) for chromosomal instability, 60.5% of the cancer patients ($n = 26$) had higher chromosomal instability compared with 11.1% of FDRs ($n = 3$) and of 12.9% of the healthy controls ($n = 4$) ($P < 0.01$). We found no difference between FDRs (11.1%) and control subjects (12.9%) ($P > 0.05$). From these findings, spontaneous MN levels in buccal cells may not be a risk indicator of inherited susceptibility in cancer families. As well buccal tissue may be less suitable for distinguishing between risk categories for HNC due to the fact that there has been small variation of MN in this tissue (14). Rosin and German (42) showed that a chromosome instability syndrome like Bloom syndrome heterozygotes was characterised by increased buccal MN frequencies. Rajeswari *et al*.(19) suggested that FDRs of breast cancer patients are at an increased cancer risk based on buccal MN frequencies and alkaline comet assay as basal DNA damage in lymphocytes. These results are not consistent with our findings. However, these authors also emphasised that follow-up studies in high-risk individuals as well as animals are required to confirm their results (19).

In the present study, analysis of MN frequencies in buccal cells from patients having HNC revealed an increased chromosomal instability, similar to the ones in the lymphocytes, although the extent of damage varied between two tissues, thus meaning that similar genotoxic events may cause MN occurrence in both tissues (39). Thus, it seems that MN frequencies in buccal cells might be sensitive to detect genetic instability related to HNC status. However, it requires prospective studies to verify these results. The similarity between the chromosomal damage in two different tissues, e.g. lymphocytes and exfoliated buccal cells, might provide a clue for the use of buccal MN as a marker of cancer risk. We did not observe any correlation between MN frequencies in lymphocytes and those in buccal cells for cancer patients. In this paper, since other nuclear anomalies had not been scored, further discussion on the predictive value of this assay could not be possible. The cytokinesis blocked MN cytome assay/buccal cytome assay is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity, thus, nuclear anomalies other than MN can lead to a better understanding of the mechanisms involved in sensitivity to chemical exposures (43,44). In fact, the comprehensive MN cytome approach both in lymphocytes and buccal cells has increasingly been adapted for the last few years (45–47).

Based on the results of our study, we also suggest that chromosomal instability as elevated spontaneous MN frequencies in lymphocytes may be used as a risk indicator for the future development of cancer in healthy relative, however, it is not case for buccal MN frequencies. Buccal tissue may be less suitable for risk categorization related to the HNC.

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References

1. Sturgis, E. M., Wei, Q. and Spitz, M. R. (2004) Descriptive epidemiology and risk factors for head and neck cancer. *Semin. Oncol.*, **31**, 726–733.
2. Mao, L., Hong, W. K. and Papadimitrakopoulou, V. A. (2004) Focus on head and neck cancer. *Cancer Cell*, **5**, 311–316.
3. Baez, A. (2008) Genetic and environmental factors in head and neck cancer genesis. *J Environ. Sci.Health C Environ. Carcinog. Ecotoxicol. Rev.*, **26**, 174–200.
4. Boyle, P. and Levin, B. (eds.) (2008) *IARC, World Cancer Report.* Lyon, France, http://www.iarc.fr/en/publications/pdfs-onliner/wcr/2008/pdf,wcr_2008.pdf (accessed May 25, 2010).
5. Copper, M. P., Jovanovic, A., Nauta, J. J., Braakhuis, B. J., de Vries, N., van der Waal, I. and Snow, G. B. (1995) Role of genetic factors in the etiology of squamous cell carcinoma of the head and neck. *Arch. Otolaryngol. Head Neck Surg.*, **121**, 157–160.
6. Foulkes, W. D., Brunet, J. S., Sieh, W., Black, M. J., Shenouda, G. and Narod, S. A. (1996) Familial risks of squamous cell carcinoma of the head and neck: retrospective case-control study. *Br. Med. J.*, **313**, 716–721.
7. Koch, W. M. and McQuone, S. (1997) Clinical and molecular aspects of squamous cell carcinoma of the head and neck in the nonsmoker and nondrinker. *Curr. Opin. Oncol.*, **9**, 257–261.
8. Garavello, W., Foschi, R., Talamini, R. *et al.* (2008) Family history and the risk of oral and pharyngeal cancer. *Int. J. Cancer.*, **122**, 1827–1831.
9. Beckman, R. A. and Loeb, L. A. (2005) Genetic instability in cancer: theory and experiment. *Semin. Cancer Biol.*, **15**, 423–435.
10. Fenech, M. (2002) Biomarkers of genetic damage for cancer epidemiology. *Toxicology*, **181**, 411–416.
11. Bonassi, S., Znaor, A., Ceppi, M. *et al.* (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28**, 625–631.
12. Murgia, E., Ballardin, M., Bonassi, S., Rossi, A. M. and Barale, R. (2008) Validation of micronuclei frequency in peripheral blood lymphocytes as early cancer risk biomarker in a nested case-control study. *Mutat. Res.*, **639**, 27–34.
13. Martins, R. A., Gomes, G. A., Aguiar, O., Jr and Ribeiro, D. A. (2009) Biomonitoring of oral epithelial cells in petrol station attendants: comparison between buccal mucosa and lateral border of the tongue. *Environ. Int.*, **35**, 1062–1065.
14. Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech, M. (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. *Mutat. Res.*, **659**, 93–108.
15. Nersesyan, A. K. and Adamyan, R. T. (2004) Micronuclei level in exfoliated buccal mucosa cells of patients with benign and malignant tumors of female reproductive organs and breast. *Tsitol. Genet.*, **38**, 72–75.
16. Celik, A., Cavaş, T. and Ergene-Gözükara, S. (2003) Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. *Mutagenesis*, **18**, 417–421.
17. Burgaz, S., Erdem, O., Cakmak, G., Erdem, N., Karakaya, A. and Karakaya, A. E. (2002) Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers*, **7**, 151–161Erratum in: Biomarkers, (2006) 11(4):383.
18. Burgaz, S., Karahalil, B., Bayrak, P., Taşkin, L., Yavuzaslan, F., Bökesoy, I., Anzion, R. B., Bos, R. P. and Platin, N. (1999) Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. *Mutat. Res.*, **439**, 97–104.
19. Rajeswari, N., Ahuja, Y. R., Malini, U., Chandrashekar, S., Balakrishna, N., Rao, K. V. and Khar, A. (2000) Risk Assessment in first degree female relatives of breast cancer patients using the alkaline comet assay. *Carcinogenesis*, **21**, 557–561.
20. Fenech, M. (1993) The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat. Res.*, **285**, 35–44.
21. Eastmond, D. A. and Tucker, J. D. (1989) Identification of aneuploidy-inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody. *Environ. Mol. Mutagen.*, **13**, 34–43.
22. Sarto, F., Finotto, S., Giacomelli, L., Mazzotti, D., Tomanin, R. and Levis, A. G. (1987) The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis*, **2**, 11–17.
23. Tolbert, P. E., Shy, C. M. and Allen, J. W. (1992) Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat. Res.*, **271**, 69–77.
24. Bonassi, S., Ugolini, D., Kirsch-Volders, M., Strömberg, U., Vermeulen, R. and Tucker, J. D. (2005) Human population studies with cytogenetic biomarkers: review of the literature and future prospectives. *Environ. Mol. Mutagen.*, **45**, 258–270.
25. Hagmar, L., Bonassi, S., Strömberg, U., Brøgger, A., Knudsen, L. E., Norppa, H. and Reuterwall, C. (1998) Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res.*, **58**, 4117–4121.
26. Duffaud, F., Orsiere, T., Digue, L., Villani, P., Volot, F., Favre, R. and Botta, A. (1999) Micronucleated lymphocyte rates from head-and-neck cancer patients. *Mutat. Res.*, **439**, 259–266.
27. Delfino, V., Casartelli, G., Garzoglio, B., Scala, M., Mereu, P., Bonatti, S., Margarino, G. and Abbondandolo, A. (2002) Micronuclei and p53 accumulation in preneoplastic and malignant lesions of the head and neck. *Mutagenesis*, **17**, 73–77.
28. Palyvoda, O., Polańska, J., Wygoda, A. and Rzeszowska-Wolny, J. (2003) DNA damage and repair in lymphocytes of normal individuals and cancer patients: studies by the comet assay and micronucleus tests. *Acta Biochim. Pol.*, **50**, 181–190.
29. Iarmarcovai, G., Ceppi, M., Botta, A., Orsière, T. and Bonassi, S. (2008) Micronuclei frequency in peripheral blood lymphocytes of cancer patients: a meta-analysis. *Mutat. Res.*, **659**, 274–283.
30. Minicucci, E. M., Kowalski, L. P., Maia, M. A., Pereira, A., Ribeiro, L. R., de Camargo, J. L. and Salvadori, D. M. (2005) Cytogenetic damage in circulating lymphocytes and buccal mucosa cells of head-and-neck cancer patients undergoing radiotherapy. *J. Radiat. Res. (Tokyo)*, **46**, 135–142.
31. Negri, E., Boffetta, P., Berthiller, J. *et al.* (2009) Family history of cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Int. J. Cancer*, **124**, 394–401.
32. Egeli, U., Ozkan, L., Tunca, B., Kahraman, S., Ceçener, G., Ergül, E. and Engin, K. (2000) The relationship between genetic susceptibility to head and neck cancer with the expression of common fragile sites. *Head Neck*, **22**, 591–598.
33. Berg-Drewniok, B., Weichenthal, M., Ehlert, U., Rümmelein, B., Breitbart, E. W. and Rüdiger, H. W. (1997) Increased spontaneous formation of micronuclei in cultured fibroblasts of first-degree relatives of familial melanoma patients. *Cancer Genet. Cytogenet.*, **97**, 106–110.
34. Battershill, J. M., Burnett, K. and Bull, S. (2008) Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies. *Mutagenesis*, **23**, 423–437.
35. Bonassi, S., Fenech, M., Lando, C. *et al.* (2001) HUman MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ. Mol. Mutagen.*, **37**, 31–45.
36. Desai, S. S., Ghaisas, S. D., Jakhi, S. D. and Bhide, S. V. (1996) Cytogenetic damage in exfoliated oral mucosal cells and circulating lymphocytes of patients suffering from precancerous oral lesions. *Cancer Lett.*, **109**, 9–14.
37. Saran, R., Tiwari, R. K., Reddy, P. P. and Ahuja, Y. R. (2008) Risk assessment of oral cancer in patients with pre-cancerous states of the oral cavity using micronucleus test and challenge assay. *Oral Oncol.*, **44**, 354–360.
38. Bloching, M., Hofmann, A., Lautenschlager, C., Berghaus, A. and Grummt, T. (2000) Exfoliative cytology of normal buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN-assay. *Oral. Oncol.*, **36**, 550–555.
39. Ceppi, M., Biasotti, B., Fenech, M. and Bonassi, S. (2010) Human population studies with the exfoliated buccal micronucleus assay: statistical and epidemiological issues. *Mutat. Res.*, **705**, 11–19.
40. Karahalil, B., Karakaya, A. E. and Burgaz, S. (1999) The micronucleus assay in exfoliated buccal cells: application to occupational exposure to polycyclic aromatic hydrocarbons. *Mutat. Res.*, **442**, 29–35.

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41. Bonassi, S., Biasotti, B., Kirsch-Volders, M. *et al*. (2009) HUMNXL Project Consortium. State of the art survey of the buccal micronucleus assay—a first stage in the HUMN(XL) project initiative. *Mutagenesis*, **24**, 295–302.
42. Rosin, M. P. and German, J. (1985) Evidence for chromosome instability in vivo in Bloom syndrome: increased numbers of micronuclei in exfoliated cells. *Hum. Genet*., **71**, 187–191.
43. Fenech, M. (2007) Cytokinesis-block micronucleus cytome assay. *Nat. Protoc*., **2**, 1084–1104.
44. Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech, M. (2009) Buccal micronucleus cytome assay. *Nat Protoc*., **4**, 825–837.
45. El-Zein, R. A., Fenech, M., Lopez, M. S., Spitz, M. R. and Etzel, C. J. (2008) Cytokinesis-blocked micronucleus cytome assay biomarkers identify lung cancer cases amongst smokers. *Cancer Epidemiol. Biomarkers. Prev*., **17**, 1111–1119.
46. Thomas, P., Hecker, J., Faunt, J. and Fenech, M. (2007) Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. *Mutagenesis*, **22**, 371–379.
47. Thomas, P., Harvey, S., Gruner, T. and Fenech, M. (2008) The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. *Mutat. Res*., **638**, 37–47.

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