ORAL DISEASES

Oral Diseases (2009) 15, 499–504. doi:10.1111/j.1601-0825.2009.01576.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Micronuclear and sister chromatid exchange analyses in peripheral lymphocytes of patients with oral lichen planus – a pilot study*

S Ergun[1], S Warnakulasuriya[2], N Duman[3], A Saruhanoğlu[1], B Sevinç[4], Ş Öztürk[5], S Özel[6], K Çefle[5], Ş Palanduz[5], H Tanyeri[1]

[1]*Faculty of Dentistry, Department of Oral Medicine and Surgery, Istanbul University, Istanbul, Turkey;* [2]*Department of Oral Medicine, King's College Hospital Dental Institute, London, UK;* [3]*Department of Genetics, Institute of Health Science, Istanbul University, Istanbul, Turkey;* [4]*Medical Genetics Programme at the Institute of Health Sciences, Istanbul University, Istanbul, Turkey;* [5]*Department of Internal Medicine Division of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey;* [6]*Department of Public Health, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey*

OBJECTIVE: The purpose of this study was to determine the genetic instability of peripheral blood lymphocytes from patients diagnosed with oral lichen planus (OLP) by investigation of frequencies of micronuclei (MN) and sister chromatid exchange (SCE).

MATERIALS AND METHODS: A total of 22 newly diagnosed and untreated patients with OLP of same severity scores and twenty healthy controls participated in this study. They were all non-smokers with no previous history or family history of cancer. The periodontal status, flow rate and buffering capacity of whole mouth saliva were recorded. SCE and MN analyses were performed on peripheral blood lymphocytes of OLP patients and healthy controls.

RESULTS: The frequencies of MN (50.00 ± 22.36) and SCE (6.89 ± 1.48) in OLP patients were found to be significantly elevated compared with that in normal individuals (25.20 ± 9.52 and 5.93 ± 1.31; $z = 3.946$, $P = 0.0001$; $z = 2.346$, $P = 0.019$). There were no significant differences in the MN frequency and SCE between the two subgroups with reticular or erosive types of OLP.

CONCLUSION: These pilot data indicate an increased genomic instability in peripheral blood lymphocytes of a cohort of Turkish patients diagnosed with oral lichen planus as compared with that of healthy individuals. As patients with OLP may have an increased or potential risk for oral malignancy, these assays could be used in translational research to monitor beneficial effects of interventions and long-term prognosis.

Oral Diseases (2009) **15**, 499–504

Correspondence: Dr S Ergun, Department of Oral Medicine and Surgery, Faculty of Dentistry, Istanbul University. Tel: +90 212 414 20 00, Fax: +90 212 531 22 30, E-mail: sertanergun@gmail.com
Received 27 March 2009; revised 23 April 2009; accepted 26 April 2009
*This study was presented as poster presentation in the 9th Biennial Congress of the European Association of Oral Medicine. September 18–20, 2008 Salzburg, Austria.

Keywords: oral lichen planus; sister chromatid exchange; micronuclei

Introduction

Lichen planus is a chronic autoimmune, inflammatory condition which can affect the skin, oral mucosa (oral lichen planus, OLP), genital mucosa, scalp and nails (Al-Hashimi *et al*, 2007). In this cell-mediated autoimmune disease of unknown aetiology, T lymphocytes accumulate in the lamina propria beneath the epithelium of the oral mucosa (Scully *et al*, 2000; Epstein *et al*, 2003). There are various clinical forms recognized: reticular, papular, plaque, atrophic, erosive (ulcerative) and bullous types that can occur separately or simultaneously (Scully and Carrozzo, 2008). Based on an age-standardized estimate, the overall prevalance of OLP in a Swedish population was reported to be 1.27% (0.96% in men and 1.57% in women) (Axell and Rundquist, 1987). It occurs mostly in the fifth to sixth decades of life, and is twice more common in women than in men (Carrozzo and Gandolfo, 1999; Saran *et al*, 2008). Malignant transformation of OLP has been reported in a number of studies, with the frequency ranging from 0% to 12.5% (Gonzalez-Moles *et al*, 2008).

Cytogenetic tests have often been applied to determine whether the presence of chromosome instability is related to the predisposition to a disease (Emingil *et al*, 2002). Among several available tests, sister chromatid exchange (SCE) and micronuclear (MN) analyses in peripheral blood lymphocytes are well-established techniques aiming to evaluate the extent of cytogenetic damage (Latt and Schreck, 1980; Latt *et al*, 1980; Heddle *et al*, 1991; Kamboj and Mahajan, 2007). SCE is a crossover of chromatin, with exchange of material between sister chromatids that occurs during cell division (Latt *et al*, 1980). Micronuclei, on the other hand,

are small extranuclei separated from the main one, generated during cellular division by whole chromosomes or by chromosome fragments (Heddle *et al*, 1991). The advantage of these tests lies in its simplicity, as the scoring of SCE and MN is rapid and is not resource intensive. Few studies have investigated the chromosomal instability of patients diagnosed with OLP (Desai *et al*, 1996; Buajeeb *et al*, 2007; Saran *et al*, 2008). By undertaking MN analysis of exfoliated oral mucosal cells, two research groups in US and India have recently demonstrated genotoxic damage in OLP patients and that their frequency is greater in the OLP group compared with that in healthy controls (Buajeeb *et al*, 2007; Saran *et al*, 2008). An earlier Indian study had shown that increased cytogenetic damage is not only present in exfoliated oral mucosal cells, but also in circulating lymphocytes of patients with OLP. Both MN and SCE analyses were used in this later study (Desai *et al*, 1996).

The aim of this study was to evaluate the chromosomal instability in patients with OLP by investigation of frequencies of MN and SCE in peripheral blood lymphocytes.

Materials and methods

Patient selection

A total of 22 patients with OLP (mean age: 44,18 ± 6.25 years; range 27 to 64 years) and twenty healthy individuals (mean age: 45,50 ± 4.48 years; range 31 to 67 years) participated in this study. Patients attending a specialist Oral Medicine Clinic in Istanbul, Turkey, with OLP diagnosed according to WHO diagnostic criteria (WHO Collaborating Centre for Oral Precancerous Lesions, 1978), were invited to participate in the study. Those meeting additional study-specific inclusion and exclusion criteria (Table 1) were enrolled. The control group (HC) consisted of age, gender-matched healthy volunteer patients who met the inclusion criteria of the study, attending other dental departments of the same institution. Informed written consent was obtained prior to recruitment from both cases and controls, and the study protocol was approved by the Institutional Ethics Committee.

Table 1 Inclusion and exclusion criteria for the selection of study participants

Inclusion criteria
Patients clinically diagnosed with oral lichen planus (OLP) confirmed by histopathology
Newly diagnosed patients prior to any treatment
OLP patients with clinical severity score 2 or below (according to Buajeeb *et al*, 2007)
Exclusion criteria
Patients with lichenoid lesions associated with drugs or restorations (Al-Hashimi *et al*, 2007
Smokers or alcohol misusers
Patients with a history of malignancy
Patients with a history of malignancy among the first-degree relatives
Patients reporting any infections within 3 months of the study
Patients who recieved periodontal therapy in the 3 months prior to the study
Exposure to cytotoxic chemicals, drugs or radiation therapy which are known to affect SCE and MN frequencies
Patients with confirmed systemic diseases (e.g. diabetes and liver disease)

Periodontal examination

Periodontal examination included bleeding on probing (BOP) (expressed as the % of sites which bled upon gentle probing), Approximal Plaque Index (API) (expressed as the % of sites which had visible plaque), probing pocket depth (PPD) measuring the distance from the crest of the gingival margin to the base of the pocket and number of the teeth present (NT) in the mouth. BOP, API and PPD were measured at four sites of each tooth; buccal, mesial, lingual and distal. All measurements were performed by the same examiner using a Hu-Friedy periodontal probe with Williams markings with a diameter of 0.5 mm.

Salivary assays

Whole unstimulated saliva was collected in a sterilized plastic tube for five minutes. The salivary flow rate (SFR) was recorded as ml min^{-1}. Salivary buffer capacity (BC) was estimated according to the procedure described by Ericson and Bratthall (Ericson and Bratthall, 1989).

SCE and MN analyses

Sister chromatid exchange and MN analyses were performed in the cytogenetic laboratory of Istanbul University, Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, using the micro-culture technique developed by Moorhead *et al* (1960). A 0.5 ml of heparinized blood obtained from patients and controls was added to the culture medium containing RPMI 1640 (Biochrom KG, Berlin, Germany), supplemented with 10% foetal calf serum, 1% PHA (Merck AG, Darmstadt, Germany) and 1% Gentamycin. At 24 h, 0.5 μg ml^{-1} 5′-bromodeoxyuridine (BrdU; Sigma Chemical Company, St Louis, USA) was added to the medium and further incubated under dark conditions. Colchicine (0.2 μ ml^{-1}; Colchicine powder; Sigma Chemical Co.) was added at 70 h. Then, cells were collected at 72 h and treated with 0.075 M KCl at 37°C for 10 min, fixed with methanol–acetic acid (Merck, Darmstadt, Germany) and standard harvest procedure was performed. Slides were stained by Florescein plus Giemsa technique. Terminal changes were counted as one, interstitial changes as two exchanges. A total of 30 well-spread metaphases with clearly visible chromosomes were scored for every subject. The frequencies of SCE per metaphase were computed.

Statistical analyses

Statistical analyses were performed by using the chi-square (Yates) test Mann–Whitney *U*-test and Sperman's rho coefficient. Statistical significance was considered as $P < 0.05$.

This study was established under approval of the Ethic Committee of Istanbul University, Faculty of Medicine. No: 2007/1316.

Table 2 The periodontal status, salivary flow rate, buffering capacity values of patients with Oral Lichen Planus (OLP-P) and healthy controls (HC)

	OLP-P (n = *22*) *mean ± SD median (IQR)*	*HS* (n = *20*) *mean ± SD median (IQR)*	*z/P**
No. teeth (NT)	17.32 ± 8.50 20.50 (6.63)	26.85 ± 1.53 28.00(1.00)	4.588/0.0001**
Bleeding on probing (BOP; %)	24.61 ± 26.96 17.07 (22.99)	15.79 ± 15.98 11.54(6.99)	0.448/0.654
Approximal Plaque Index (API; %)	32.70 ± 33.20 27.10 (26.64)	25.02 ± 24.76 14.28(14.59)	0.407/0.684
Probing pocket depth (PPD; mm)	1.46 ± 0.39 1.35 (0.25)	1.32 ± 0.27 1.26(0.17)	0.920/0.358
Salivary flow rate (SFR; ml min^{-1})	0.44 ± 0.22 0.40 (0.11)	0.55 ± 0.27 0.50(0.20)	1.322/0.186
Buffering capacity (BC; ph)	4.64 ± 0.66 4.50 (0.50)	4.78 ± 0.44 5.00(0.25)	1.086/0.278

*Mann–Whitney *U*-test; **statistically significant.

Table 3 Sister chromatid exchange (SCE) and micronuclear (MN) frequencies of the patients with Oral Lichen Planus (OLP-P) and healthy controls (HC)

	OLP-P (n = *22*) *mean ± SD median (IQR)*	*HS* (n = *20*) *mean ± SD median (IQR)*	*z/P**
Sister chromatid exchange (SCE) frequency (per metaphasis)	6.89 ± 1.48 6.67 (0.70)	5.93 ± 1.31 5.88 (0.79)	2.346/0.019**
Micronucleus (MN) frequency (in 1000 cells)	50.00 ± 22.36 50.00 (11.88)	25.20 ± 9.52 23.00 (6.38)	3.946/0.0001**

*Mann–Whitney *U*-test; **statistically significant.

Results

A total of 22 patients with OLP (12 female and 10 male) and 20 healthy subjects (nine female and 11 male) were enrolled in this study. No statistically significant differences were observed between the study and the control groups in terms of age and gender ($P > 0.05$). Out of the 22 OLP patients, there were 17 with reticular form (77%) and five with erosive form (23%).

Oral clinical examination revealed no significant differences between OLP patients and HC with regard to BOP, API, PPD, SFR and BC (Table 2, $P > 0.05$). A significant difference was, however, noted in the number of remaining teeth present ($P = 0.0001$). BC was significantly different between patients with reticular (4.82 ± 0.64) and erosive (7.87 ± 2.30) forms in the OLP group ($P = 0.007$), while no other significant differences were found between the two types of OLP patients with regard to evaluated periodontal parameters (NT: 17.82 ± 8.96 and 15.60 ± 7.30; BOP: 24.11 ± 28.23 and 26.10 ± 25.71; API: 36.36 ± 36.93 and 21.71 ± 16.15; PPD: 1.40 ± 0.33 and 1.63 ± 0.53) and SFR (0.41 ± 0.20 and 0.54 ± 0.28; $P > 0.05$).

Mean frequencies of SCE and MN were significantly different between OLP patients and HC (Table 3, $P < 0.05$), while no significant differences were observed between the subgroups of OLP patients with reticular or erosive form ($P > 0.05$). Mean MN scores were significantly different when compared by gender of patients in the OLP group ($P = 0.021$), while no differences were observed in the HC group ($P = 0.087$). There were no significant gender differences either in OLP patients or in the HC group in the mean estimates of SCE ($P > 0.05$). There were no significant correlations between the mean frequencies of SCE and MN between either OLP patients ($r_s = 0.247$, $P = 0.267$) or the HC group ($r_s = -0.053$, $P = 0.826$).

Discussion

Oral lichen planus is one of the common inflammatory oral mucosal disorders affecting the oral cavity (McCartan and Healy, 2008). The likelyhood of malignant transformation of OLP has been reported in the literature and many studies have suggested that OLP may be a potentially malignant disorder (van der Meij *et al*, 2003; Warnakulasuriya *et al*, 2007). However, the statistical probability between OLP and oral squamous cell carcinoma (OSCC) is still unknown. Chronic inflammation, ulceration and tissue damage have been associated with cancers at various sites, and oral lichen planus is a good model to examine elevated chromosomal instability and neoplastic evolution (Epstein *et al*, 2003).

Sister chromatid exchange analysis is a sensitive cytomolecular technique and has applications in the evaluation of genotoxicity and genetic instability (German and Alhadeff, 2001; Bayani and Squire, 2005). High frequency of SCE is regarded as a biomarker of early mutagenic effect suggesting increased risk of cancer (Kamboj and Mahajan, 2007). Evaluation of MN frequency was developed especially to investigate

X-ray-induced genotoxicity (Countryman and Heddle, 1976). Micronuclei may arise from acentric chromosomes and chromatid fragments in proliferating cells. When a cell with such chromosomal damage divides, these fragments are excluded from the main nucleus and form membrane-bound micronuclei in the daughter cells (Heddle *et al*, 1991). Based on preliminary evidence collected from 10 countries, a significant increase of cancer incidence was found for subjects with an increased MN frequency in preipheral blood lymphocytes (Bonassi *et al*, 2007).

Frequency of SCE could be affected by many factors such as smoking, drug use, exposure to chemotherapeutic agents, etc. These confounding factors were controlled in this study by careful medical interviews. Rowland and Harding (1999) reported that SCE frequencies of the smokers and ex-smokers are remarkably higher than the SCE frequencies of the non-smokers. For this reason, we excluded any smokers from both groups but the likelyhood of passive smoking could not be established.

Sister chromatid exchange frequencies of the healthy individuals whose family members developed malignancy were reported to be statistically higher than those with no family history of cancer (Ghidoni *et al*,1984; Parkes *et al*, 1985). Increased SCE frequency in young women with breast cancer and in their first-degree relatives has also been reported (Cefle *et al*, 2006). Therefore, our control group was selected among people with no family history of cancer.

Aggressive and severe periodontal diseases are additional confounding factors that could affect SCE and MN frequencies (Emingil *et al*, 2002; Bloching *et al*, 2008). Oral clinical examination of subjects and control groups in our study revealed mild or moderate periodontal diseases and none had severe periodontitis. The disease severity was comparable in the two groups. This is consistent with the results of a Spanish study which reported that no significant differences were observed between patients with OLP and healthy controls, with reference to periodontal indices (Ramón-Fluixá *et al*, 1999).

There are several environmental factors that could contribute to elevated SCE frequency. Among them are some preservatives in human or animal foods, especially in canned foods, that are known to effect SCE frequency (Brogger *et al*, 1989; Borda *et al*, 1996; Bozsakyova *et al*, 2001). There is no reason why data in the OLP group should be influenced by these environmental factors in a different way to our control group.

One limitation of this study was the small number of the patients included in the analysis. This reflects the difficulty of finding newly diagnosed, untreated, non-smoking subjects to avoid confounding effects that could influence the sensitive parameters that were being investigated in this study. Although the sample size was small, this study aimed to ascertain whether or not a selected cohort of patients with oral lichen planus showed evidence of genetic instability. The data show a highly significant difference ($P = 0.019$) between the mean SCE in the experimental group (6.89 ± 1.48) and the mean of the healthy control group (5.93 ± 1.31). The OLP group also had highly significantly different mean MN count, which was twice that of the control group ($P = 0.0001$).

Micronuclei formation and SCE are events biologically relevant to carcinogenesis. These estimates are quantitative reflections of chromosomal damage. To our knowledge, this is the first European investigation of oral lichen planus that indicates an increased risk towards susceptibility to develop cancer by using these biomarker tests in circulating lymphocytes. Limitation of the study is that any chromosomal damage was not assessed in the tissues from the affected site, but it was carried out in accessible body fluids. Ideally, these studies should be conducted on the target tissue. It is likely that any damage by the chronic inflammatory process to epithelial cells stimulates apoptotic cell death and reactive epithelial hyperproliferation that could promote further mutation (Kim *et al*, 2001; Philip *et al*, 2004; Moss and Blaser, 2005). On the other hand, some studies have confirmed comparable data on these biomarker measurements in both circulating lymphocytes and buccal epithelial cells both in OLP and in other environmental genotoxic studies (Desai *et al*, 1996; Lucero *et al*, 2000). Furthermore, Hagman *et al* (1994) have pointed out that the extent of genetic damage in peripheral blood lymphocytes reflects critical events for any carcinogenic processes in the target tissue.

Increased SCE and MN counts have been demonstrated in few other potentially malignant disorders of the oral cavity and in head and neck malignancies (Gadhia *et al*, 2005). A high frequency of MN has been reported among Indian tobacco chewers (Stich *et al*, 1982a,b), betel quid chewers (Gandhi and Kaur, 2000) and in oral submucous fibrosis (Cox and Walker, 1996). Our investigation complements previous studies that examined predisposition to malignancy in OLP by estimating chromosomal damage using tests that are simple and inexpensive and could be performed *in vitro*. These data suggest that OLP patients have increased SCE and MN levels in peripheral lymphocytes, which are surrogate markers for DNA damage indicative of potential for malignancy in OLP. A reduction in micronuceli in OLP patients during supplementation with beta-carotene was recently reported (Buajeeb *et al*, 2008) and is thus a helpful marker for evaluation of the disease during interventions. MN assay in buccal epithelial cells as a tool for biomonitoring DNA damage has been proposed (Holland *et al*, 2008). The ongoing human micronucleus project (http://www.humn.org) may be a useful resource for researchers interested in OLP if biomarker data could be collected from patients diagnosed with OLP to serve in translational programmes.

In conclusion, our data indicate that MN and SCE evaluation may have potential as an intermediate tool for assessing any cancer risk in OLP. These additional biomarker assays along with clinicopathological parameters could be tested in prospective studies surveying prognostic and predictive markers during clinical studies.

Author contributions

S Ergun and Ş Öztürk have designed the study. S Ergun and A Saruhanoglu made the clinical investigations. N Duman, B Sevinç, K Çefle and Ş Palandüz made the laboratory investigations. S Özel made the statistical analyses. S Ergun, S Warnakulasuriya, Ş Öztürk and H Tanyeri wrote the paper.

References

Al-Hashimi I, Schifter M, Lockhart PB *et al* (2007). Oral lichen planus and oral lichenoid lesions: diagnostic and therapeutic considerations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103:** 1–12.

Axell T, Rundquist L (1987). Oral lichen planus: a demographic study. *Coomun Dent Oral Epidemiol* **15:** 52–56.

Bayani J, Squire JA (2005). Sister chromatid exchange. *Curr Protoc Cell Biol* **22:** Unit 22.7.

Bloching M, Reich W, Schubert J, Grummt T, Sandner A (2008). Micronucleus rate of buccal mucosal epithelial cells in relation to oral hygiene and dental factors. *Oral Oncol* **44:** 220–226.

Bonassi S, Znaor A, Ceppi M, Chang WP, Holland N *et al* (2007). An increased micronuclei frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis* **28:** 625–631.

Borda E, Camusso JJ, Perez Leiros C *et al* (1996). Circulating antibodies against neonatal cardiac muscarinic acetylcholine receptor in patients with Sjogren's syndrome. *Mol Cell Biochem* **164:** 335–341.

Bozsakyova E, Chalupa I, Sebova L, Slamenova D (2001). Chromosomal aberrations, sister chromatid exchanges and micronuclei induced by pentoxifylline in in vitro cultivated Chinese hamster cells (V79) and human blood lymphocytes. *Mutat Res* **18:** 63–70.

Brogger A, Kolmannskog S, Nicolaysen RB, Wesenberg F, Nygaard R (1989). Structural chromosome aberrations in lymphocytes from children previously treated for Wilms' tumor or Hodgkin's disease. *Pediatr Hematol Oncol* **6:** 95–103.

Buajeeb W, Kraivaphan P, Amornchat C, Triratana T (2007). Frequency of micronucleated exfoliated cells in oral lichen planus. *Mutat Res* **5:** 191–196.

Buajeeb W, Kraivaphan P, Amornchat C, Suthamajariya K (2008). Reduction of micronuclei in oral lichen planus supplemented by beta-carotene. *J Oral Sci* **50:** 461–467.

Carrozzo M, Gandolfo S (1999). The management of oral lichen planus. *Oral Dis* **5:** 196–205.

Cefle K, Ucur A, Guney N *et al* (2006). Increased sister chromatid exchange frequency in young women with breast cancer and in their first-degree relatives. *Cancer Genet Cytogenet* **1:** 65–67.

Countryman PI, Heddle JA (1976). The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutat Res* **41:** 321–332.

Cox S, Walker M (1996). Oral submucous fibrosis. *Aust Dent J* **41:** 294–299.

Desai SS, Ghaisas SD, Jakhi SD, Bhide SV (1996). Cytogenetic damage in exfoliated oral mucosal cells and circulating lymphocytes of patients suffering from precancerous oral lesions. *Cancer Lett* **109:** 9–14.

Emingil G, Sapmaz G, Biçakçi N, Ozkinay F (2002). Sister chromatid exchange (SCE) analysis in periodontitis. *J Clin Periodontol* **29:** 811–815.

Epstein JB, Wan LS, Gorsky M, Zhang L (2003). Oral lichen planus: progress in understanding its malignant potential and the implications for clinical management. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **96:** 32–37.

Ericson D, Bratthall D (1989). Simplified method to estimate salivary buffer capacity. *Scand J Dent Res* **97:** 405–407.

Gadhia PK, Vaniwala S, Pithawala M (2005). Some observations on spontaneous sister chromatid exchange frequencies and cell cycle progression in stimulated lymphocytes of patients with different malignancies. *Int J Hum Genet* **5:** 187–191.

Gandhi G, Kaur R (2000). Cytogenetic studies in exfoliated cells of high cancer risk groups, pan masala chewers. *Hum Ecol* **9:** 21–228.

German J, Alhadeff B (2001). Analysis of sister-chromatid exchanges. *Curr Protoc Hum Genet* **8:** Unit 8.6.

Ghidoni A, Privitera E, Raimondi E, Rovini D, Illeni MT, Cascinelli N (1984). Sister chromatid exchange in phytohemagglutinin-stimulated lymphocytes of nonfamilial cutaneous malignant melanoma patients. *Basic Life Sci* **29:** 855–858.

Gonzalez-Moles MA, Scully C, Gil-Montoya JA (2008). Oral lichen planus: controversies surrounding malignant transformation. *Oral Dis* **14:** 229–243.

Hagman L, Broger A, Hansteen IL, Heim S *et al* (1994). Cancer risk in human predicted by levels of chromosomal abberations in lymphocyets: Nordic study group on the health risk of chromosomes damage. *Cancer Res* **54:** 2919–2922.

Heddle JA, Cimino MC, Hayashi M *et al* (1991). Micronuclei as an index of cytogenetic damage: past, present, and future. *Environ Mol Mutagen* **18:** 277–291.

Holland N, Bolognesi C, Kirsch-Volders M *et al* (2008). he 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.

Kamboj M, Mahajan S (2007). Micronucleus – an upcoming marker of genotoxic damage. *Clin Oral Investig* **11:** 121–126.

Kim J, Yook JI, Lee EH *et al* (2001). Evaluation of premalignant potential in oral lichen planus using interphase cytogenetics. *J Oral Pathol Med* **30:** 65–72.

Latt SA, Schreck RR (1980). Sister chromatid exchange analysis. *Am J Hum Genet* **32:** 297–313.

Latt SA, Schreck RR, Loveday KS, Dougherty CP, Shuler CF (1980). Sister chromatid exchanges. *Adv Hum Genet* **10:** 267–331.

Lucero L, Pastor S, Suárez S *et al* (2000). Cytogentic biomonitoring of Spanish greenhouse workers exposed to pesticides; micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. *Mutat Res* **464:** 2555–2562.

McCartan BE, Healy CM (2008). The reported prevalence of oral lichen planus: a review and critique. *J Oral Pathol Med* **37:** 447–453.

van der Meij EH, Schepman KP, van der Waal I (2003). The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **96:** 164–171.

Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerfort DA (1960). Chromosomes preparations of leucocytes cultured from human peripheral blood. *Exp Cell Res* **20:** 613.

Moss SF, Blaser MJ (2005). Mechansms of disease: inflammation and origins of cancer. *Nat Clin Pract Oncol* **2:** 90–97.

Parkes DJ, Scott D, Stewart A (1985). Changes in spontaneous SCE frequencies as a function of sampling time in lymphocytes from normal donors and cancer patients. *Mutat Res* **3:** 113–122.

Philip M, Rowley DA, Schreiber H (2004). Inflammation as a tumour promoter in cancer induction. *Semin Cancer Biol* **14:** 433–439.

Ramón-Fluixá C, Bagán-Sebastián J, Milián-Masanet M, Scully C (1999). Periodontal status in patients with oral lichen planus: a study of 90 cases. *Oral Dis* **5:** 303–306.

Rowland RE, Harding KM (1999). Increased sister chromatid exchange in the peripheral blood lymphocytes of young women who smoke cigarettes. *Hereditas* **2:** 143–146.

Saran R, Tiwari RK, Reddy PP, Ahuja YR (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.

Scully C, Carrozzo M (2008). Oral mucosal disease: lichen planus. *Br J Oral Maxillofac Surg* **46:** 15–21.

Scully C, Eisen D, Carrozzo M (2000). Management of oral lichen planus. *Am J Clin Dermatol* **1:** 287–306.

Stich HF, Curtis JR, Parida BB (1982a). Application of micronuclear test to exfoliated cells of high cancer risk groups: tobacco chewers. *Int J Cancer* **30:** 553–559.

Stich HF, Stich W, Parida BB (1982b). Elevated frequency of micronucleated cellsin the buccal mucosa of individuals at high risk from oral cancer: betel quid chewers. *Cancer Lett* **17:** 125–134.

Warnakulasuriya S, Johnson NW, van der Waal I (2007). Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med* **36:** 575–580.

WHO Collaborating Centre for Oral Precancerous Lesions (1978). Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol* **46:** 518–539.