Chromosomal Numerical Aberrations in Oral Lichen Planus

INTRODUCTION

Oral lichen planus (OLP), a cell-mediated immune condition, affects the oral mucosa in approximately 1-2% of the population (Epstein et al., 2003). Clinically, OLP presents in several forms, mainly reticular, atrophic, and erosive. The malignant potential of OLP has been the subject of controversy in the literature. Several studies reported an annual malignant transformation risk of 0.04%-1.74% (van der Meij et al., 1999; Lodi et al., 2005). In contrast, others claim that the association of OLP and oral squamous cell carcinoma (OSCC) is either coincidental or represents misdiagnosis of dysplastic epithelial lesions (lichenoid dysplasia) as OLP (Mithani et al., 2007).

To uncover some of the molecular genetic aberrations associated with OLP, investigators have used molecular and genetic methods such as loss of heterozygosity (LOH) (Zhang et al., 2000), chromosome in situ hybridization (Kim et al., 2001), p53 and c-erbB2 expression (Girod et al., 1994, 1995; Kilpi et al., 1996; Ogmundsdottir et al., 2002), and telomerase activity (O’Flatharta et al., 2002). However, none of these methods appears to provide definitive predictive information with regard to the malignant potential of a lesion diagnosed as OLP.

Alteration in the number of chromosomes, defined as aneuploidy, stands out as the most consistent marker of malignancy and is the earliest and most distinctive pre-neoplastic genotypic (Galipeau et al., 1996; Duesberg and Li, 2003; Rajagopalan and Lengauer, 2004; Erenpreisa et al., 2005). In head and neck cancer, and particularly in OSCC, the incidence of aneuploidy is high (up to 80% of cases) (Stell, 1991; Milroy et al., 1997; Wennerberg et al., 1998; Bockmühl and Petersen, 2002) and has been found to be an early event in humans as well as in animal models (Remmerbach et al., 2001; Maraki et al., 2004; Raimondi et al., 2005; Pektas et al., 2006). Kim et al. (2001), using chromosomal in situ hybridization, found a certain extent of genetic instability for chromosome 9 in OLP. Nevertheless, the study used biopsy-based material and is therefore not suitable for clinical follow-up, where a non-invasive approach is required.

Recently, a combined morphological and FISH analysis at the single-cell level was introduced (Shimoni et al., 2002). This method facilitates the distinction of small populations of aneuploid cells among overlapping diploid cells, thereby enhancing the specificity of pathological cell detection (Kaplinsky et al., 2003). We have recently examined oral brush samples to detect aneuploid cells in oral leukoplakias (Hirshberg et al., 2007). The proportion of aneuploid cells increased with the degree of severity of the histopathologic diagnosis; aneuploid cells were detected even in lesions diagnosed as epithelial hyperplasia or mild dysplasia.

The present study aimed to evaluate the presence of chromosomal numerical aberrations in cells collected by brush sampling from persons with OLP by combined morphological and FISH analysis. The results were investigated for a possible correlation with the clinical outcome.
MATERIALS & METHODS

Study Population

The study group included 57 consecutive persons with OLP referred to the Oral Medicine Clinic at the Department of Oral and Maxillofacial Surgery, the Chaim Sheba Medical Center, for the diagnosis and management of OLP.

Selected were only those with a definite clinical diagnosis of OLP based on the WHO diagnostic criteria (1978), namely, presence of a bilateral lace-like network of slightly raised gray-white lines (reticular pattern); erosive, atrophic, bullous, and plaque-type lesions were accepted as a subtype only in the presence of reticular lesions elsewhere. In all cases, the diagnosis of OLP was confirmed by histopathologic examination demonstrating hyperortho-hyperparakeratosis, degeneration of the basal layer, and subepithelial lymphocytic band-like infiltrate (WHO, 1978). All cases were subjected to study following clinical and histopathologic diagnosis.

Data including information on age, gender, signs and symptoms, sites of involvement, and the clinical form of OLP (reticular, atrophic, and erosive) were collected. Thirty-three healthy individuals with no history of tobacco or alcohol use and with normal-looking oral mucosa served as the control group.

Persons who failed to meet the above criteria, or those with an identifiable cause, such as a hypersensitivity to dental restorative materials or drugs, were excluded from the study.

Consent was obtained from all participants in accordance with a protocol approved by the Institutional Review Board for Clinical Studies at the Sheba Medical Center, and by the Ministry of Health for the use of genetic material.

Sample Collection from the Oral Cavity

Oral samples were collected by means of a disposable brush with nylon fibers, measuring 2.5 x 5 mm, small enough to ensure homogeneous sampling with light rotational movements. The brush was placed in RPMI 1640 medium containing 10% FCS (Gibco, Invitrogen, Carlsbad, CA, USA).

Two samples were taken from each person: from an area affected by lichen planus and from normal-looking mucosa at a different oral site, preferably, whenever possible, at the opposite site. In the control individuals, 2 samples were taken from the posterior lateral border of the tongue and from the buccal mucosa.

Combined Analysis of Morphology and I-FISH Experiments

The technical procedures involving the Duet™ system (Bioview Ltd., Rehovot, Israel) have already been described (Trakhtenbrot et al., 2002). Briefly, cytopsin preparations were air-dried and stained with May Grunwald-Giemsa (Sigma, St. Louis, MO, USA). Slides were scanned by the bright-field mode of the system, based on a dual-mode, fully automated microscope (Axioplan2, Carl Zeiss, Jena, Germany), fitted with an XY motorized stage with an accuracy of 0.2 µm (Marzhauser, Wetzlar, Germany), and a 3CCD progressive scan camera (DXC9000, Sony, Tokyo, Japan). The stain was removed by methanol/acetic acid (3:1), and FISH was performed on the same slides by standard procedures (Cohen et al., 2000). The system enables the fluorescent signals in the specific cells to be observed with a x100 objective (magnification x1000) simultaneous to the morphology of the same cells scanned previously. Cells with bright signals were scanned manually and captured as target nuclei. Dual-color FISH with alpha satellite probes for the chromosome #2 labeled with Spectrum Green and chromosome #8 labeled with SpectrumOrange (Vysis, Downer’s Grove, IL, USA) was performed. In normal nuclei, the hybridization pattern produces 2 red /2 green signals. We defined a cell as aneuploid if one of the probes showed more than 2 signals. FISH with an alpha satellite probe for chromosome #9 labeled with SpectrumGreen was performed in cases with abnormal hybridization patterns of chromosomes #2 and #8.

The parallel analysis of morphology and FISH facilitates a clear distinction between epithelial and inflammatory cells and between overlapping cells which may mimic false polyploidy. The percentage of cells with more than 2 signals of chromosomes #2, #8, and #9 from the entire cell population was calculated for each case.

The selection of chromosomes #2 and #8 was based on the results of our previous study (Hirshberg et al., 2007), in which we used these chromosomes successfully to detect aneuploid cells in oral leukoplakias and squamous cell carcinomas. Chromosome #9 was selected following the study conducted by Kim et al. (2001), who found a certain extent of genetic instability for chromosome #9 in OLP.

Statistical Analysis

Data were analyzed by SPSS statistical software, version 14.0 (SPSS Inc., Chicago, IL, USA). We used the chi-square test to compare the prevalence of aneuploid cells and demographic and clinical data in both study groups. Odds with 95% confidence intervals were computed from these regression results.

RESULTS

In each of the 33 control individuals, 100-500 cells were examined. In two people, 1.3% and 1.5% of the cells were aneuploid, i.e., a cut-off value (mean values ± 3 SD) of aneuploid cells was determined as 1.1%, with a specificity of 93.9% (P = 0.013).

In the study group, aneuploid cells comprising over 1.1% of the examined cells were found in 16 people (28.1%) (Table 1); this was referred to as the positive group. Persons with less than 1.1% aneuploid cells were considered negative. In all but three people (Nos. 9 and 15 in the positive group and one from the negative group), more than 100 cells (average, 213 cells) were examined.

The mean age of the study group was 55 yrs, with a 1:2 male-to-female ratio. Twenty-three percent were smokers, and only two persons were alcohol consumers (Table 2). Comparison of the positive and negative groups disclosed only a few differences. In the negative group, there was female predominance, and the percentage of smokers was higher; however, these differences did not reach statistical significance. The reticular form was the most common clinical presentation (over 50%) in both groups.
Combined FISH and Morphological Analysis

Analysis of the cells obtained from the clinically affected mucosa revealed aneuploid cells over 1.1% in 16 persons (28.1%) (Table 1, Fig.). In 10 individuals, over 5% of the cells were aneuploid (17.5%), and in two (Nos. 1 and 2), most of the cells were aneuploid. In seven (12.3%) persons (Nos. 1, 2, 6, 8, 9, 10, and 15), aneuploid cells comprising over 1.1% of the examined cells were found in the samples collected from the apparently normal-looking mucosa.

Variations existed in the hybridization patterns of the 2 chromosomal probes. Six people in the positive group exhibited numerical aberrations of both chromosomes. However, in only two persons (Nos. 4, 13) was there an equal number of signals of chromosomes #2 and #8 in all the cells examined; in the remaining 14 persons, unequal numbers of signals were detected. In six persons, aberrations were found only with chromosome #8, and in four people, only with chromosome #2.

Differences in the numbers of signals existed between cells from the same samples—for example, in participant No. 7, 2% of the cells showed equal gain of both chromosomes (3 green/3 red), while 8% showed gain of only chromosome #2 (3 green/2 red), and in participant No. 2, 3 different populations of cells were found. Analysis of the sample from participant No. 1 revealed an extreme heterogeneity, with 8 different hybridization patterns. All but one participant were normal for chromosome #9. In participant No. 1, extra signals or chromosome #9 was found only in those cells expressing gains of chromosomes #2 and #8. All the examined aneuploid cells had normal morphology when cytomorphology was compared with FISH.

No correlation was found between gender, smoking habits, clinical presentation, and the proportion of aneuploid cells detected in the positive group.

Follow-up

The follow-up period, in most cases, was short; in 52 persons, it was fewer than 18 mos, and in four persons, over 36 mos. One person (No. 6) developed an invasive cancer in a follow-up period of 30 mos. Interestingly, the carcinoma developed at the site where the control sample was collected and contained 8% aneuploid cells.

DISCUSSION

Most persons with OLP are asymptomatic, and once the diagnosis is established, the persons are either watched or ignored. It is therefore a matter of utmost importance to identify a subgroup of high-risk individuals for follow-up. About a quarter of the participants in the present study exhibited aneuploid cells in their oral brush samples. Aneuploid cells were also detected in the normal-looking mucosa in seven individuals, one of whom developed an invasive cancer at that site, which is not unexpected when one considers that lichen planus is a diffuse non-static condition where lesions wax and wane over time. Although uncommon, tumors have been reported to occur also in areas of mucosa which clinically appeared to be non-involved by OLP (Mignogna et al., 2007).

Controversy exists over whether the concept of field cancerization should be extended to OLP, which is considered to have malignant potential unrelated to common risk factors (e.g., tobacco and alcohol usage) (Gandolfo et al., 2004). Recently, it has been suggested that the process of field cancerization is not necessarily associated with field exposure to environmental carcinogens, but describes the frequent occurrence of multiple primary tumors in epithelial areas affected by a widespread pre-malignant disease (Ha and Califano, 2003). The presence of aneuploid cells in the affected and non-affected oral mucosa found in the present study supports the concept of field cancerization in OLP; moreover, persons with OLP were found to develop multiple and multifocal neoplastic events, an occurrence which parallels the process of field cancerization of oral cancer (Mignogna et al., 2007).
Table 1. Clinical, Histopathology, and FISH Results of the Positive Group of Participants

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, yrs/ Gender</th>
<th>Tobacco</th>
<th>Clinical Diagnosis</th>
<th>No. of Cells Examined</th>
<th>Aneuploid Cells, %</th>
<th>3 Green/ 2 red, %</th>
<th>3 Red/ 2 Green, %</th>
<th>3 Green/ 3 Red, %</th>
<th>4 Green/ 4 Red, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60/F No RLP</td>
<td>300</td>
<td>76.3</td>
<td>Normal</td>
<td>250 11.6</td>
<td>8 39.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>59/F No ELP</td>
<td>200</td>
<td>73.5</td>
<td>Normal</td>
<td>200 53.5 50</td>
<td>3.5 10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>61/F No ALP</td>
<td>260</td>
<td>36</td>
<td>Normal</td>
<td>300 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>65/M No ALP</td>
<td>200</td>
<td>30</td>
<td>Normal</td>
<td>200 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>66/M No RLP</td>
<td>160</td>
<td>12</td>
<td>Normal</td>
<td>400 0.5</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>62/M No RLP</td>
<td>150</td>
<td>10</td>
<td>Normal</td>
<td>200 8</td>
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<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>69/F No ELP</td>
<td>240</td>
<td>9</td>
<td>Normal</td>
<td>150 5</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>50/M No RLP</td>
<td>70</td>
<td>7</td>
<td>Normal</td>
<td>100 2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>50/M Yes RLP</td>
<td>110</td>
<td>6</td>
<td>Normal</td>
<td>100 6</td>
<td>6</td>
<td></td>
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</tr>
<tr>
<td>11</td>
<td>58/M No ELP</td>
<td>500</td>
<td>2.7</td>
<td>Normal</td>
<td>400 0.25</td>
<td>2</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>55/F No ALP</td>
<td>250</td>
<td>2.4</td>
<td>Normal</td>
<td>200 0</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>13</td>
<td>34/M No RLP</td>
<td>200</td>
<td>2</td>
<td>Normal</td>
<td>100 0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>56/F Yes ALP</td>
<td>100</td>
<td>2</td>
<td>Normal</td>
<td>100 1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>47/M No RLP</td>
<td>70</td>
<td>1.4</td>
<td>Normal</td>
<td>300 1.3</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>69/F No ELP</td>
<td>300</td>
<td>1.3</td>
<td>Normal</td>
<td>300 0</td>
<td>1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In addition - 4g/3r-4%, 4g/3r-3.4%, 5g/5r-13.4%, 5g/4r-5.3%, 6g/6r -1.4%, 6g/5r-1.4%.

M, male; F, female; RLP, reticular lichen planus; ELP, erosive lichen planus; ALP, atrophic lichen planus; green, chromosome 2 green signals; red, chromosome 8 red signals; Normal, sample from apparently healthy-looking mucosa.

Table 2. Clinical and Demographic Comparison between the Positive and Negative Groups

<table>
<thead>
<tr>
<th>Statistical Significance</th>
<th>Positive Group, N = 16</th>
<th>Negative Group, N = 41</th>
<th>Total, N = 57</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age [range, yrs]</td>
<td>56.5 (34-69)</td>
<td>55.2 (17-82)</td>
<td>55.6 (17-82)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>8 (50.0%)</td>
<td>14 (34.1%)</td>
<td>22 (38.6%)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>8 (50.0%)</td>
<td>27 (65.9%)</td>
<td>35 (61.4%)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>2 (12.5%)</td>
<td>11 (27.5%)</td>
<td>13 (23.2%)</td>
</tr>
<tr>
<td>Alcohol use (%)</td>
<td>1 (6.3%)</td>
<td>1 (2.6%)</td>
<td>2 (3.6%)</td>
</tr>
<tr>
<td>Reticular lichen planus (%)</td>
<td>8 (50.0%)</td>
<td>23 (56.0%)</td>
<td>31 (54.4%)</td>
</tr>
<tr>
<td>Erosive lichen planus (%)</td>
<td>4 (25.0%)</td>
<td>7 (17.5%)</td>
<td>11 (19.3%)</td>
</tr>
<tr>
<td>Atrophic lichen planus (%)</td>
<td>4 (25.0%)</td>
<td>11 (27.5%)</td>
<td>15 (26.3%)</td>
</tr>
<tr>
<td>Mean follow-up, months [range]</td>
<td>19.8 (13-38)</td>
<td>14.2 (6-36)</td>
<td>15.7 (6-38)</td>
</tr>
</tbody>
</table>

RLP, reticular lichen planus; ELP, erosive lichen planus; ALP, atrophic lichen planus; NS, non-significant.
Only a few cytogenetic studies have been conducted in OLP. The data obtained in these studies are in agreement with the present results, indicating that OLP may harbor chromosomal instability. Kim et al. (2001), using chromosomal in situ hybridization on paraffin-embedded tissue sections obtained from persons with OLP, found a certain extent of genetic instability for chromosome #9. We did not find any alterations in chromosome 9 in all but one individual. The differences between the studies can be explained by the different methods applied; the use of paraffin-embedded tissue sections can lead to confounding problems, such as nuclear slicing, variations of hybridization efficiency, and counting error, which may be responsible for the excess of monosomic cells. More recently, DNA aneuploidy in a subset of persons with OLP was found by image cytometry (Femiano and Scully, 2005). However, this method of determining DNA content has well-known pitfalls and difficulties that make this technique not sufficiently sensitive to guide treatment regimes for high-risk persons with OLP.

The relatively high proportion of individuals with chromosomal aberrations has also been reported (Montebugnoli et al., 2006). These investigators disclosed clonal chromosomal aberrations in about 20% of OLP persons by applying G-banding chromosome analysis to short-term primary cultures on 30 individuals. Chromosome gains can increase gene dosage of particular genes, thus leading to a selective survival advantage. The results of the present study indicate the importance of chromosomal numerical aberrations of chromosomes #2 and #8. It stands to reason, however, that additional chromosomes may be involved as well.

The present study suggests that the combined morphological and FISH analysis with brush sampling can identify a subgroup of persons for monitoring once the diagnosis is established. The simultaneous analysis is sensitive enough to detect single aneuploid cells among large populations of diploid cells and facilitates clear distinction of various cells, mainly the inflammatory cells that are so frequent in OLP, and between overlapping cells, which may mimic false polyploidy. Moreover, the examined aneuploid cells in the collected samples had normal morphology when cytomorphology was compared with FISH. Thus, the combined morphological and FISH analysis enhances the early detection of potentially malignant cells, even before cytologic changes are apparent by traditional histopathology.

Whether aneuploidy will lead to a progression to cancer is yet to be investigated. In the present study, one person developed an invasive cancer; a sample collected almost 3 yrs earlier demonstrated 8% aneuploid cells. The supplement of a brush sample and the combined morphological and FISH analysis, therefore, can increase specificity in predicting the nature of persons with OLP. Nevertheless, the observations should be strengthened by continuing follow-up and increasing the size of the study population.

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REFERENCES


