Cytological analysis of the oral cells of chronic renal failure patients: a cytomorphometric study

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Aim: The aim of this study was to cytologically analyze the tongue mucosa, buccal mucosa, and floor of the mouth of patients with chronic renal failure and healthy volunteers to determine what cellular changes are caused by uremic diseases.

Materials and methods: In order to evaluate cellular changes induced by chronic renal failure, exfoliative cytology was used for the analysis of the tongue mucosa, buccal mucosa, and floor of the mouth of 20 uremic patients with chronic renal failure and 20 healthy volunteers. Smears from each individual were stained using the Papanicolaou method and were analyzed using a stereological method.

Results: The mean cytoplasmic and nuclear geometric volume values of oral cells were measured in both groups. Statistically the nuclear volumes and cytoplasmic volumes in the dorsum tongue, buccal mucosa, and floor of the mouth epithelial cells were markedly higher in the patient group than the control group (P < 0.005).

Conclusion: These findings suggest that there are alterations in the dorsum tongue, buccal mucosa, and floor of the mouth epithelial cells in chronic renal failure, which are detectable by microscopy and cytomorphometry. Further research is needed to determine related factors.

Key words: Chronic renal failure, oral mucosa, exfoliative cytology

Introduction

Chronic renal failure (CRF) is defined as a progressive decline in renal function associated with a reduced glomerular filtration rate. CRF affects many body systems and clinical features vary according to the stage of renal failure (1). Several oral changes are seen in patients with chronic renal failure; specifically, a wide range of oral mucosal lesions, particularly white patches and/or ulceration, has been described in these patients (1,2).

Although many pathological conditions affecting the oral mucosa can be distinguished clinically, a definitive diagnosis of the lesion is often required before the commencement of appropriate treatment.

The most accepted clinical technique for the diagnosis of lesions in the oral mucosa is incisional or excisional biopsy (3). However, in specific clinical conditions, such as CRF and diabetes, many invasive techniques lose viability as a result of variations in the international normalized ratio (INR), blood glucose levels, and the disease itself (4,5). In these cases, oral exfoliative cytology may be more appropriate. This method allows a quick and fairly accurate assessment of suspicious lesions of the oral cavity. Due to the evolution of computing capabilities and quantitative techniques, morphometry can be used to evaluate the geometric features of the normal and abnormal cells present in oral smears (6).
Stereology is an important method for morphometric assessment. Stereology is the 3-dimensional interpretation of planar sections of materials or tissues, which to a large extent provides a morphometric analysis (7,8). For some smaller objects, such as cells, stereological methods exist to obtain volume parameters. These include optical fractionator with contour trace, Cavalieri estimator, surface-weighted star volume, area fraction fractionator method, and 3- or 2-dimensional nucleator (7,8).

The aim of this study was to investigate the quantitative cytological changes of oral mucosa smears collected from chronic renal failure patients undergoing dialysis, and to describe the risk of oral cancer of chronic renal failure patients in comparison with healthy control subjects.

Materials and methods
A total of 20 patients with CRF (12 men and 8 women) undergoing maintenance peritoneal dialysis therapy and 20 healthy volunteers (13 men and 7 women) were recruited from the Department of Internal Medicine, Faculty of Medicine, Atatürk University, Erzurum, Turkey. Before enrollment, each subject consented to the protocol, which was reviewed and approved by the Medical Ethics Committee of Atatürk University. A pro forma inventory was completed, detailing name, age, sex, and relevant medical history. Patients with anemia, diabetes mellitus, radiotherapy, alcohol consumption, and smoking were not included in the study.

Smears were obtained from the clinically healthy tongue mucosa, buccal mucosa, and floor of the mouth of patients with chronic renal failure and from volunteer control individuals. After examination at the clinic, the oral mucosa was dried with a gauze swab to remove surface debris and excess saliva. Smears were taken from the tongue dorsum, buccal mucosa, and floor of the mouth of 20 patients with CRF and 20 healthy volunteers using a cytobrush and transferred to clean, dry glass slides. These were then immediately sprayed with a commercial fixative containing 95% ethyl alcohol. Smears from each individual, stained by the Papanicolaou method, were analyzed using the stereological method with the nucleator in 2 dimensions.

The smears were placed on a motor-driven stage attached to a microscope and an image of the cells at 200× magnification was projected onto the monitor via camera. Each clearly defined cell with predominant staining was examined by systematic sampling in a stepwise manner, moving the microscope stage from left to right and then down and across, in order to avoid remeasuring the same cells. The nuclear volume (NV) and cytoplasmic volume (CV) were evaluated for each cell using software (Steroinvestigator, MicroBrightField).

The volume of the interesting cells obtained from buccal mucosa, tongue dorsum, and floor of the mouth of patients with CRF and healthy volunteers' smears was estimated using the nucleator method using the following formula (9) (Figures 1A–1F):

\[ V_n = \frac{4}{3} \pi l^3 \]

where

- \( V_n \) = Number weighted volume
- \( l \) = Length of intercepts
- \( n \) = Number of nucleator estimates

Although a number of requirements for obtaining full randomness in the section-sampling stage while estimating the volume of the small objects by the nucleator method were mentioned in the literature (10,11), we only implemented one requirement associated with the section stage of the sampling procedure because we used a smear.

The cytomorphometric data were compared between the patients with CRF and control groups using the independent samples t-test. The statistical analysis was performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Levels of significance were set at \( P < 0.05 \) and \( P < 0.001 \).

Results
Twenty chronic renal failure patients receiving dialysis treatment participated in this study, including 12 men and 8 women (mean age: 42.9 ± 16.2 years). At baseline, the mean time of dialysis treatment was 28.0 ± 24.6 months. The mean age was 40.21 years (standard deviation [SD]: 10.90) in the control group (13 men and 7 women).
Figure 1. Histologic view of exfoliative cell samples. Top grid presents buccal mucosa of the control (A, B, C) and dialysis patients (A1, B1, C1). Bottom grid shows exfoliate profiles obtained from the floor of the mouth of the control (D, E, F) and dialysis patients (D1, E1, F1).
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The mean cytoplasmic and nuclear geometric volume values of oral cells (tongue cytoplasmic volume (TCV), buccal cytoplasmic volume (BCV), floor of mouth cytoplasmic volume (FCV), tongue nucleus volume (TNV), buccal nucleus volume (BNV), and floor of mouth nucleus volume (FNV)) are presented in Table 1 for patients with CRF and the control group. As shown in Table 2, statistically significant differences were observed for CV and NV between groups. The nuclear area and cytoplasmic area were markedly higher (P < 0.005) in the patient group than the control group. The nucleus/cytoplasm (N/C) mean (tongue nucleus/tongue cytoplasm (TN/TC), buccal nucleus/buccal cytoplasm (BN/BC), and floor of mouth nucleus/floor of mouth cytoplasm (FN/FC)) was higher in the CRF group than the control group. However, these differences were not statistically significant (Tables 1 and 2).

**Discussion**

Oral manifestations have been reported in end-stage renal disease patients, including gingivitis, xerostomia, an ammonia-like odor resulting from a high urea content, mucosal pallor and lesions, tooth mobility, malocclusion, and an increased risk of dental erosion due to frequent regurgitation (2,12). In this study, microscopic and cytomorphometric analyses of the oral epithelium in CRF patients were performed due to the little research that has been done before on this subject. The cytomorphometric findings in the oral smears of CRF patients demonstrated that there were statistically significant differences present in the NV and CV.

Inflammatory changes of tongue mucosa in uremic patients can be monitored microscopically. The findings may be explained by the presence of superficial erosions or ulcerations of the tongue mucosa's squamous epithelium, such as in diffuse stomatitis and gingivitis (2,12,13). In this context, the squamous cells present in smears of the mucosa become partially or completely substituted by cells originating from deeper epithelial layers.

Oral mucosa of uremic patients can lead to similar changes in other factors, such as nutritional deficiencies like hypochromic anemia (iron deficiency) and megaloblastic anemia (deficiency of vitamin B12 and folic acid). Vitamin B12 and folic acid are essential substances for DNA synthesis. These 2 factors, included in nutritional deficiency, impair DNA synthesis with both the nucleus and cytoplasm increasing in size (4).

The oral cavity is cleaned by saliva, which removes food residue from the surface. If the flow of saliva diminishes considerably, the accumulation of bacteria increases in the mouth (9,14,15). It is known that uremic patients have lower salivary flow rates and that their saliva may contain urea (16,17). These factors may affect the cytomorphometric alterations of the oral mucosa cells.

Alberti et al. (4) performed cytomorphometric analyses of the oral epithelium in type II diabetic patients and found that there was a substantial increase in the nuclear area while the cytoplasmic area did not present any significant differences. They suggested that the cellular modifications may be related to an increase in inflammatory cells and

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**Table 1. The mean of TCV, BCV, FCV, TNV, BNV, and FNV values of control and study groups and statistical comparison.**

<table>
<thead>
<tr>
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<th>Control group</th>
<th>Study group</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>TCV(µm³)</td>
<td>20</td>
<td>6.9679E4 ± 18,046.2</td>
<td>20</td>
</tr>
<tr>
<td>BCV(µm³)</td>
<td>20</td>
<td>5.2391E4 ± 22,830.4</td>
<td>20</td>
</tr>
<tr>
<td>FCV(µm³)</td>
<td>20</td>
<td>6.0162E4 ± 18,795.2</td>
<td>20</td>
</tr>
<tr>
<td>TNV(µm³)</td>
<td>20</td>
<td>2.6165E2 ± 108.4</td>
<td>20</td>
</tr>
<tr>
<td>BNV(µm³)</td>
<td>20</td>
<td>2.5740E2 ± 138.6</td>
<td>20</td>
</tr>
<tr>
<td>FNV(µm³)</td>
<td>20</td>
<td>4.3620E2 ± 121.8</td>
<td>20</td>
</tr>
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P < 0.05.
mucosa keratinization in type II diabetic patients (4).

A number of factors that could influence the cytomorphology of oral mucosa cells have been investigated. These include radiotherapy, smoking, alcohol, and malignant oral lesions. Therefore, the effects of such factors, if present, should be taken into account when assessing a lesion (18–20).

Keles et al. (21) suggested that there was a real increase in the NV in the oral smears of transplant patients, as the CV present was statistically significantly different from, and the N/C mean was higher in, the transplant patient group. They suggested that oral cells may have malignant transformation in transplant patients.

Göregen et al. (22) found that the average nuclear area, nuclear perimeter, minimal nuclear diameter, and maximal nuclear diameter values of the buccal mucosa cell nuclei of smokers were higher than those of nonsmokers.

As a result of the fact that exfoliative cytology is considered a moderate and noninvasive technique compared to a conventional examination, an assessment of the application of exfoliative cytology in uremic patients is necessary to better interpret the findings of specimens obtained from the mucosa of affected patients. A good knowledge of this technique, regarding the morphological and morphometric aspects of the cells, may enable the implementation of exfoliative cytology in public health programs, which would be another useful tool for the diagnosis of CRF. We hope that if this is achieved, the diagnostic potential of exfoliative cytology in the diagnosis of early oral malignancy will be greatly enhanced. Further research is needed to determine related factors.

### References


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