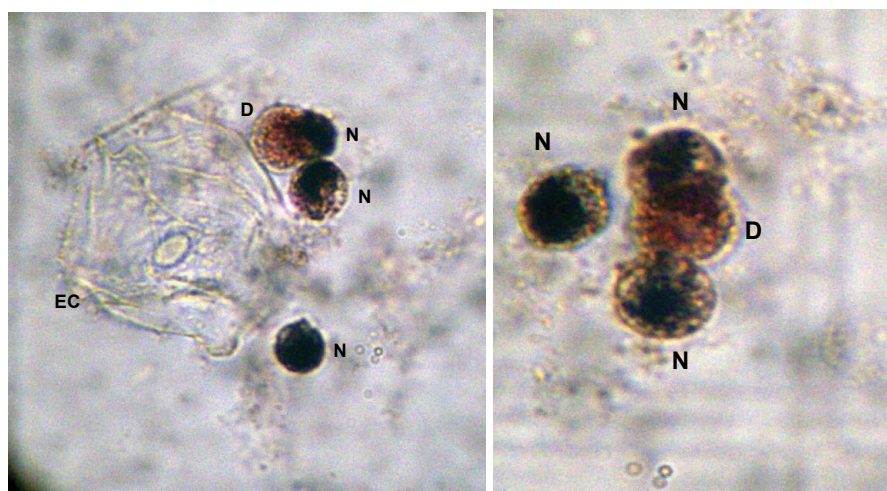


**Clinical Image****Title: Benzidine Staining - Method to Detect Neutrophils in Whole Saliva of Patients with Renal Disease****Cardoso EML<sup>1,2\*</sup>, Arregger AL<sup>1</sup>, Budd D<sup>1</sup>, Elverdin JC<sup>2</sup>, Tumilasci G<sup>2</sup> and Contreras LN<sup>1,2</sup>**<sup>1</sup>Endocrine Research Department, Instituto de Investigaciones Médicas A. Lanari, University of Buenos Aires, Unidad Ejecutora IDIM-CONICET, Buenos Aires, Argentina<sup>2</sup>Laboratories of Salivary Glands, Department of Physiology, School of Dentistry, University of Buenos Aires, Argentina

**Figure 1:** Peroxidase positive polymorphonuclear leukocytes in saliva from patients with renal disease (benzidine-staining) N: Intact neutrophils; EC: Epithelial Cell, D: Disintegrated neutrophil

**Abstract**

Benzidine staining allows for the quantification of peroxidase positive polymorphonuclear leukocytes in whole saliva from patients with renal disease. This is a practical tool for monitoring oral health in this high risk population.

**Keywords:** Salivary neutrophils; Salivary peroxidase positive polymorphonuclear leukocytes; Renal disease

Polymorphonuclear leukocytes (PMNs) are present in even the healthiest mouth. These cells migrate predominantly through gingival crevices into the oral cavity. The number of PMNs in exudate reflects the degree of gingival inflammation. However, a substantial amount of PMNs are also found in the whole saliva of edentulous subjects demonstrating the migration of these cells through the oral mucosa from the circulation. Salivary PMNs can contribute to the oral defence against infection in two major ways: phagocytosis and the release of antimicrobial proteins (lysozyme, myeloperoxidase, lactoferrin, etc.) [1].

Periodontal disease, including both gingivitis and periodontitis, are among the most widespread chronic conditions affecting populations worldwide with up to 50-70% of adults afflicted globally [2,3]. Periodontitis, the destructive form of periodontal disease is a non-reversible inflammatory disease involving the tissues that surround and support the teeth. Untreated, the disease leads to destruction of the alveolar bone that surrounds the teeth, and thus to tooth mobility and loss of dentition [4]. The inflammatory and immune reactions induced by bacterial adherence and growth on the surface of the teeth are primarily responsible for the pathogenesis of periodontitis. Destruction of tissues occurs as a result of an over-aggressive immune response with the massive release of neutrophils, reactive oxygen species and enzymes [5].

Quantification of oral neutrophils levels is important in understanding periodontal disease, and the role of these cells in it. Findings have shown that PMNs detected in biological fluids using immunohistological methods correlate well with granulocyte counts obtained using peroxidase method. The benzidine-method for the detection of peroxidase positive PMNs is quick, low-cost and useful in screening biological fluids for the presence of granulocytes [6]. The brown stained peroxidase positive granules color the cytoplasm allowing the nuclei to be distinguished by contrast. Desintegrated PMNs appear as clusters of brown granules.

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In short, the technique consists in mixing 20 µl of whole saliva with 20 µl of PBS buffer and 40 µl of benzidine working solution. This is incubated for 5 minutes at room temperature. Then, the samples are observed under a light microscope. Brown neutrophils are counted in 16 large squares of a Neubauer chamber (at a magnification of 40x) and expressed as PMNs/ml as described [6].

A high prevalence of periodontal disease is described in chronic renal failure. By using the technique described in Figure 1, we evaluated 12 patients with glomerular filtration rates between 30.0 to 90.0 ml/min/1.73 m<sup>2</sup>. Saliva samples were obtained after 2.0 hours fasting at 9.0 a.m. by having patient spit directly into sterile polypropylene tubes for 5.0 minutes. A 20 µl of whole saliva was immediately processed for benzamine PMN staining as described. We found that in 57% of patients the PMN count was more than 10<sup>6</sup> cells/ml, above the PMNs count found in healthy subjects [7].

In summary, this study demonstrated that it is possible to quantify salivary neutrophils using the benzidine technique. This could be a very useful tool for monitoring oral health in patients with renal disease.

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