

MULTIMODAL ASSESSMENT AND MANAGEMENT OF ORAL HOMOGENOUS LEUKOPLAKIA: CYTOGENETIC AND URINARY NICOTINE METABOLITE EVALUATION ALONG WITH LASER ABLATION

Medicine

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ABSTRACT

Oral leukoplakia is a potentially malignant disorder strongly associated with tobacco use and carries a risk of malignant transformation. This case report evaluated oral leukoplakia using cytological staining techniques, including Feulgen and AgNOR staining, along with biochemical assessment of urinary nicotine metabolite cotinine levels. The report also monitored treatment response following CO₂ laser ablation and low-level laser therapy. A 60-year-old male patient presenting with homogeneous leukoplakia of the right buccal mucosa underwent CO₂ laser ablation. Cytological analysis was performed to assess micronuclei frequency and cellular proliferative activity, while urinary cotinine estimation was used to evaluate systemic tobacco exposure. Postoperative healing was satisfactory, with no evidence of recurrence during follow-up. Cytogenetic staining demonstrated evidence of genetic damage and increased cellular activity, whereas urinary cotinine analysis confirmed continued systemic exposure to tobacco. The combined use of laser therapy, cytogenetic markers, and biochemical assessment provided a comprehensive approach for diagnosis, risk evaluation, treatment, and monitoring of oral leukoplakia.

KEYWORDS

Oral Leukoplakia; Micronucleus; AgNOR; Urinary Cotinine; CO₂ Laser; Low-level Laser Therapy.

INTRODUCTION

Oral leukoplakia (OL) is a white lesion with malignant potential, identified after excluding other oral disorders that are also white [1,2]. OL has a prevalence rate among the Indian population which varies between 0.2-5.2% [3]. The abuse of tobacco, either smoked or smokeless, is the commonest aetiological factor [1,4,5]. Numerous studies have shown a very close relationship between tobacco usage and both the induction and malignant transformation of leukoplakia [4-6]. Cytogenetic methods such as Micronucleus Assay and Argyrophilic Nucleolar Organizer Region (AgNOR) staining are powerful aids for detecting chromosomal damage, genomic changes, and increased cell proliferative activity associated with oral potentially malignant disorders [7,8]. These are early markers for genotoxic damage detection and offer a method for assessing the biological effect of treatment or preventive modalities [7,8]. Additionally, assessment of cytogenetics alone does not provide a comprehensive analysis. Biochemical measurement of tobacco-related damage is also an important component [8]. Urinary cotinine serves as a biomarker for assessing tobacco exposure and related damage [8]. The combined application of Feulgen-AgNOR staining with urinary cotinine estimation provides a broader framework for multimodal assessment [7,8]. Whereas Feulgen-AgNOR staining depicts abnormalities at cellular and nuclear levels, urinary cotinine measurement reflects the intensity of systemic tobacco exposure [7,8]. This approach may help establish meaningful correlations between tobacco consumption and genotoxic damage, enhance individual risk stratification, and facilitate early diagnosis and timely intervention among populations with a high prevalence of tobacco consumption [3,5,8]. Promising results with lower recurrence rates and reduced postoperative morbidity have emerged with modern therapeutic modalities such as carbon dioxide (CO₂) laser ablation compared with conventional surgical excision. CO₂ laser therapy has demonstrated effective local control in a high proportion of cases [9,10].

Despite previous studies concerning the use of cytogenetic markers as well as urinary cotinine in oral leukoplakia separately, there is inadequate information on their simultaneous utilization to evaluate the therapeutic effectiveness in CO₂ laser ablation. The purpose of this research is to assess the variations in the number of micronuclei, AgNORs, and urinary cotinine concentrations before and after CO₂ laser ablation in patients suffering from oral leukoplakia.

Case Study

A 60-year-old male presented to the Department of Oral Medicine and Radiology with a chief complaint of a mobile crown prosthesis involving teeth 11 and 21. The patient's medical history revealed diabetes mellitus and hypertension diagnosed six months earlier, for which he was receiving appropriate medications. He also reported a history of tobacco use in both smoked and smokeless forms, with an average frequency of three to four times daily. Written informed consent was obtained prior to clinical valuation and treatment.



Figure 1: Clinical Presentation of Homogeneous Oral Leukoplakia.

Clinical intraoral photograph showing a unilateral, diffuse, non-scrapable white plaque with a characteristic "cracked mud" appearance involving the right buccal mucosa. The lesion extends from the labial commissure to the retromolar region along the occlusal plane and appears slightly elevated with a rough surface, consistent with a provisional diagnosis of homogeneous oral leukoplakia.

An incisional biopsy procedure was deferred because of the Homogenous clinical findings, the patient's choice, systemic comorbidities, and the planned laser ablation procedure. Cytogenetic studies are utilized as ancillary non-invasive tests; however, the gold standard still is histopathology.

Exfoliative cytology using Feulgen and AgNOR staining was subsequently performed to evaluate micronuclei frequency and cellular proliferative activity.

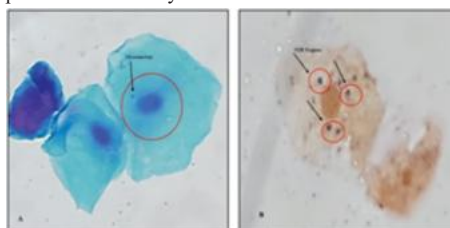


Figure 2: Cytogenetic Assessment of Buccal Epithelial Cells Using Feulgen and AgNOR Staining

A) Feulgen-stained buccal epithelial cell showing a micronucleus (arrow); pre-treatment MN frequency: 9/1000 cells. (B) AgNOR-

stained buccal epithelial cell showing argyrophilic nucleolar organizer regions (arrows); pre-treatment mean AgNOR count: 3/nucleus ($\times 1000$, oil immersion).

Quantitative comparisons of pre- and post-treatment micronuclei scores, AgNOR counts, and urinary nicotine/cotinine levels are summarized in Tables 1-3.

Table – 1 Feulgen Micronuclei (MN) Score: Comparison of Feulgen-stained Micronuclei (MN) Score Before and After Treatment. The Micronuclei Frequency Decreased from 9 MN/1000 Cells in the Pre-Treatment Sample to 3 MN/1000 Cells in the Post-Treatment Sample, Indicating a Reduction in Genotoxic Damage Following Treatment.

Parameter	Pre-Treatment	Post-Treatment
MN/1000 cells	9	3

Table – 2 AgNOR Regions Score: Comparison of AgNOR Score Before and After Treatment. The Mean Number of Argyrophilic Nucleolar Organizer Regions (AgNORs) Per Nucleus Decreased From 3 in the Pre-Treatment Sample to 1 in the Post-Treatment Sample, Indicating a Reduction in Cellular Proliferative Activity Following Treatment.

Parameter	Pre-Treatment	Post-Treatment
Mean NOR/ Nucleus	3	1

Table – 3 Urinary Metabolite Nicotine/Cotinine Analysis (CLIA Method) Comparison of Urinary Nicotine/Cotinine Metabolite Levels (Measured by Chemiluminescent Immunoassay [CLIA]) Before and After Treatment. The Pre-treatment Urinary Nicotine/Cotinine Concentration was >500 ng/mL, Which Decreased to <500 ng/mL Following Treatment, Indicating a Reduction in Tobacco/Nicotine Exposure.

Parameter	Value (ng/mL)
Pre-Treatment	>500
Post-Treatment	<500

The patient's management consisted of initial conservative therapy followed by CO₂ laser ablation, postoperative low-level laser therapy, and serial follow-up evaluations. A summary of the clinical course and treatment timeline is presented in Table 4.

Table–4 Clinical Course and Treatment Timeline

Time Point	Clinical Intervention and Findings
Day 0	Clinical examination, baseline intraoral photograph, exfoliative cytology (Feulgen MN and AgNOR staining), urinary nicotine/cotinine estimation (CLIA), and tobacco cessation counseling were performed.
Days 7–14	Topical clotrimazole 1% w/v and topical retinoid therapy were prescribed, followed by clinical reassessment showing persistence of the lesion.
Day 21	CO ₂ laser ablation of the lesion was performed under local anesthesia, followed immediately by low-level laser therapy (LLLT).
Post-operative Days 24–28	The ablation site demonstrated satisfactory healing with symptomatic relief and a characteristic whitish healing scar, following which topical vitamin E was prescribed.
Week 4	Repeat exfoliative cytology and urinary nicotine/cotinine estimation demonstrated reduced MN frequency, AgNOR count, and urinary cotinine levels.
Six Months	Clinical follow-up showed near-complete resolution of the lesion without recurrence, with residual physiological pigmentation and continued tobacco cessation counseling.

Postoperative review during Days 24-28 demonstrated satisfactory healing of the laser-treated site with formation of a characteristic whitish healing scar and no evidence of secondary infection



Figure 3: Postoperative Healing of the Lesion on Days 24–28

Clinical intraoral photograph obtained on postoperative Days 24–28 demonstrating satisfactory healing of the laser-ablated lesion. The treated site exhibited a characteristic whitish healing scar following laser ablation, with no evidence of secondary infection.

At the six-month follow-up visit, the lesion showed near-complete clinical resolution without recurrence, with only residual physiological pigmentation remaining.



Figure 4: Six-Month Follow-up Intraoral Photograph

Intraoral photograph obtained at 6 months following CO₂ laser ablation, demonstrating progressive regression of the leukoplakic lesion with restoration of normal mucosal architecture. At 6 months, near-complete clinical resolution of the lesion was evident, with residual physiological pigmentation at the treated site.

DISCUSSION

Oral leukoplakia is the most frequent among the oral potentially malignant disorders, and solely based on its clinical presentation it is difficult to predict the likelihood of transformation into malignancy. Additional diagnostic methods evaluating genetic modifications, cellular proliferation and exposure to tobacco could contribute to the evaluation of risks and therapy control [1,2,5].

Micronucleus test is an extensively used cytogenetic method to evaluate chromosomal injury and genomic instability in orally exfoliated epithelial cells. In the current case, increased pre-treatment micronuclei rate (9 MN/1000 cells) could suggest genetic damage linked to tobacco use. Decrease in the number of micronuclei following CO₂ laser therapy and smoking cessation counseling (3 MN/1000 cells) could be viewed as a result of decreased genotoxic effects. The same tendency was observed in studies evaluating the cytogenetic profile in patients with oral potentially malignant disorders utilizing Feulgen staining and micronucleus detection [6,7]. However, exfoliative cytology could be regarded as an additional diagnostic method only, while histopathologic examination remains the "gold standard" of diagnosis and evaluation of epithelial dysplasia [1,2].

AgNOR staining serves as an indirect measurement of cell proliferative capacity by revealing argyrophilic nucleolar organizer regions within the nuclei. The high pre-treatment AgNOR value of 3 per nucleus indicated high levels of epithelial proliferative capacity, while its reduction post-treatment to 1 per nucleus indicated normalization of cellular kinetics as a result of treatment. There have been previous research studies that show increased AgNOR numbers in precancerous oral lesions [6].

In contrast to cytogenetic markers, measurement of urinary cotinine indicates nicotine intake of a patient on a systemic level. Increased pre-treatment urinary cotinine value (>500 ng/mL) indicated nicotine intake despite counseling. Even though it decreased post-treatment to <500 ng/mL, further testing is needed because urinary cotinine is a biochemical indicator of nicotine intake and treatment adherence [8].

CO₂ laser ablation has proved to be a successful mode of treatment for oral leukoplakia due to its precision, good hemostasis, little postoperative pain, and good wound healing ability. In this case, satisfactory wound healing was obtained without any occurrence of secondary infection or recurrence in the six months following period. These results agree with previous works that have found favorable healing and low recurrence rates in cases where CO₂ laser was used to treat oral leukoplakia [9,10].

The main strength of this paper is that it includes the simultaneous use

of the clinical examination along with the genetic and biochemical analyses (Feulgen micronucleus test and AgNORs staining in addition to measurement of urinary cotinine before and after the CO₂ laser ablation). Although each of these procedures separately has been studied in oral leukoplakia, there is a lack of papers that integrate the three methods to assess the effect of laser treatment on patients with this disease.

However, there are certain limitations to this current report. The report describes only one clinical case of patient, who was under follow-up observation for six months. The histopathologic examination was not performed, which implies that the diagnosis of epithelial dysplasia could not be done. Moreover, individual variations during the process of cytologic examination have to be taken into account when the results of the study are being analysed.

CONCLUSIONS

The case illustrates the usefulness of combining clinical assessment with cytogenetic analysis (Feulgen micronucleus test and AgNOR staining) and biochemical testing via urinary cotinine in the overall management of oral leukoplakia. CO₂ laser ablation combined with low level laser therapy was found to be effective in terms of clinical healing, decrease in cytogenetic changes and urine cotinine levels during the follow-up period. This combination of different methods of investigation is informative and needs to be studied further.

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