



## Diagnostic Reliability of Fine Needle Aspiration Cytology against Histopathology for the Diagnosis of Oral Squamous Cell Carcinoma and Oral Leukoplakia

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### ABSTRACT

**Aim:** The purpose of this study was to determine the possibility of using fine needle aspiration cytology (FNAC) as a primary diagnostic test in oral leukoplakia and squamous cell carcinoma.

**Materials and methods:** This study consisted of clinically diagnosed 15 cases of leukoplakia and 15 cases of oral squamous cell carcinomas. FNAC and biopsy were done on all the cases. A cytological and histopathological correlation was undertaken to determine the proportion of cancers. A 23-gauge sterile disposable needle was attached to a disposable syringe and introduced into the lesion at the proposed biopsy site in one movement. In leukoplakias, the center of the lesion or erythroplakic areas and, in squamous cell carcinomas, proliferative areas and edges of the ulcers were chosen.

**Results:** In leukoplakia group, out of 15 biopsy samples, one (6.67%) sample was negative and 14 (93.33%) were positive. Whereas out of 15 FNAC samples, 14 (93.33%) were negative and one (6.67%) sample was positive. In squamous cell carcinoma, out of 15 biopsy samples, no sample was negative and all (100.00%) were positive. Whereas out of 15 FNAC samples, two (13.33%) were negative and 13 (86.67%) sample were positive.

**Conclusion:** It is noted that FNAC can be employed as a sound diagnostic tool for rapid diagnosis of oral squamous cell carcinoma. It may be particularly useful in cases, where formal biopsy procedure is difficult or contraindicated due to medical reasons or in cases of advanced malignancy.

**Clinical significance:** FNAC has been shown to be reliable and safe technique in the diagnosis of malignant in the head and neck. When the aspirations are performed by cytopathologists, it is easy to perform a rapid staining of the first smear and within 10 to 15 minutes to ensure that the material is sufficient and diagnosable and to suggest a preliminary diagnosis.

**Keywords:** Oral carcinoma, Squamous, Fine needle aspiration cytology, Histopathological examinations.

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### INTRODUCTION

A variety of aids or adjuncts to the diagnosis of oral precancerous and cancerous lesions have been developed over the years, several within the past decade. Although primarily developed for use by the general dental practitioner, data have been published to suggest possible utility in the hands of specialists as well. As with any test, proper case selection and correct performance of the test itself are critical to the sensitivity and specificity of its result.<sup>1,18</sup>

The role of Fine needle aspiration cytology (FNAC) in diagnosis of oral cancer at earlier stages has been ignored, even though it is safe, simple, economic, well tolerated, relatively pain less and an ambulatory procedure. It also carries little risk or stress to the patient. FNAC has been shown to be reliable and safe technique in the diagnosis of malignancies in head and neck region. It has been employed extensively in the study of lymph nodes, salivary glands and bone lesions.<sup>1,12-14,16,17</sup>

When the aspirations are performed by cytopathologists, it is easy to perform a rapid staining of the first smear and within 10 to 15 minutes to ensure that the material is sufficient and diagnosable and to suggest a preliminary diagnosis.<sup>1,5-10</sup>

### MATERIALS AND METHODS

The study included clinically diagnosed 15 cases of oral squamous cell carcinomas and 15 cases of oral leukoplakia, attending the dental outpatient ward of New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh.

The procedure was well explained to the patients and due consents were taken from all of them. All patient

received local anesthesia in the form of lignocaine spray or lignocaine viscous gargle to avoid gag reflex and pain. The samples were taken with the patients in a supine or sitting position with a head support. A 23-gauge sterile disposable needle was attached to a disposable syringe and introduced into the lesion at the proposed biopsy site in one movement. 'In leukoplakias, the center of the lesion or erythroplakic areas were chosen (Fig. 1).' 'In squamous cell carcinomas, proliferative areas and edges of the ulcers were chosen (Fig. 2).' The needle was gently moved in different directions, while a steady suction was applied to the piston. It was undesirable to draw the material into the syringe. The piston was allowed to fall back until the pressure was equalized. The puncture site was covered with gauze and firm pressure applied.<sup>3,4</sup>

'Proposed biopsy site of mild-to-severe dysplasia showing highly variable, ranging from hyperkeratosis and hyperplasia to atrophy and severe dysplasia (Fig. 3).' 'And, biopsy site of squamous cell carcinoma of paraffin section stained with hematoxylin and eosin (H&E) demonstrates

cords of epithelial cells infiltrating the underlying tissue, irregular cords and nests of pleomorphic epithelial cells, and the presence of concentrically arranged epithelial cells with formation of keratin pearls (Fig. 4).'

The needle was detached and air was drawn into the syringe. The needle was reattached and the aspirate was ejected onto pre-labeled slides. The syringe contents were emptied quickly on two slides and excess blood was absorbed using gauze pads. The residual tissue fragments were transferred to another clean slide before the onset of clot formation. Smears were made by spreading the aspirate with another slide, similar to drawing a blood smear. If there was semisolid material on the specimen slide, the spreader slide was turned over and the flat surface was pressed gently on these tiny fragments and then the slides were pulled apart to result in evenly spaced cells on both slide surfaces.<sup>11</sup>

The slides were wet fixed with 1:5 ether alcohol. Two slides were prepared for each patient. One slide was stained with H&E and another one slide with rapid papanicolaou and traut (PAP) stain.<sup>1,2,12-15</sup>



Fig. 1: Clinical photograph of leukoplakia

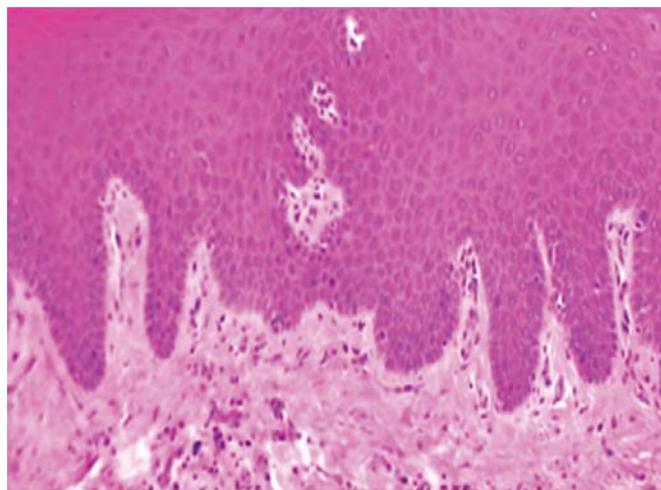


Fig. 3: Histologic feature of oral leukoplakia



Fig. 2: Clinical photograph of oral squamous cell carcinoma

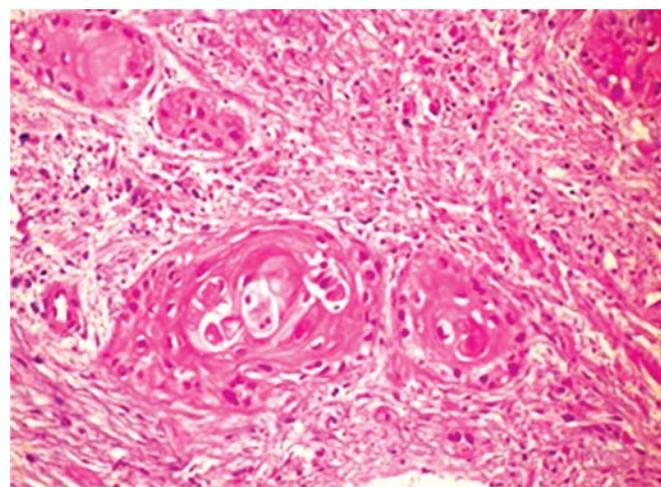


Fig. 4: Histologic feature of well-differentiated squamous cell carcinoma

An incisional biopsy was performed at the site of FNAC. Formalin-fixed, paraffin-embedded specimens were then sectioned to 0.5  $\mu$ m thickness and stained with routine H&E stain.<sup>6-8</sup>

FNAC and histopathologic reports of these patients were reviewed to find out the accuracy of FNAC with the HPE report.

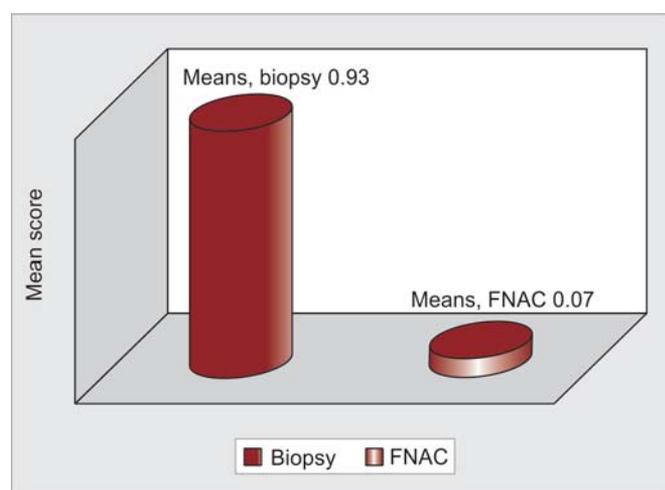
The results were tabulated and analyzed.

## RESULTS

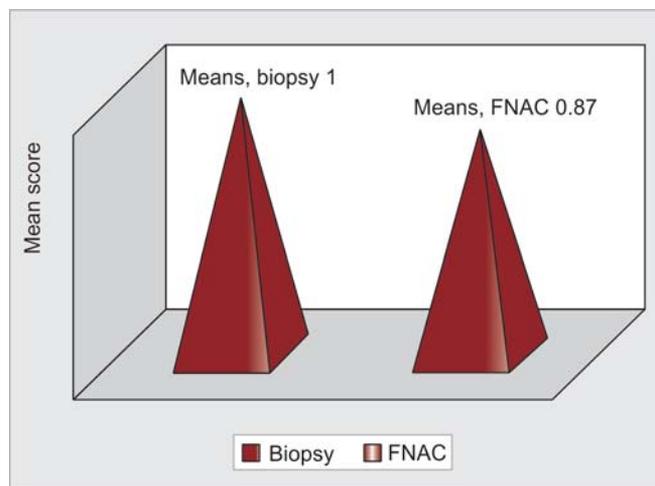
The study group consisted of clinically diagnosed 15 cases of leukoplakia and 15 cases of oral squamous cell carcinomas. In leukoplakia group, out of 15 biopsy samples, one (6.67%) sample was negative and 14 (93.33%) were positive. Whereas out of 15 FNAC samples, 14 (93.33%) were negative and one (6.67%) sample was positive (Graph 1). Mean scores of gradings leukoplakia, according to the groups.

In squamous cell carcinoma, out of 15 biopsy samples, no sample was negative and all (100.00%) were positive. Whereas out of 15 FNAC samples, two (13.33%) were negative and 13 (86.67%) samples were positive (Graph 2). Mean scores of squamous cell carcinoma, according to the study groups.

In oral leukoplakia, equal rates of true-positive and false-negative results were obtained. The overall positive correlation accounted for 93.33% with 93.33% inadequate FNAC'S. In squamous cell carcinomas, the specificity was 100.00% with a predictive value of 53.37% (when all the obtained FNAC reports were compared with histopathology reports) and it was found that in 14 samples of leukoplakia group, where FNAC showed negative results were turned out to positive histopathologically.



**Graph 1:** Mean scores of gradings (leukoplakia) according to groups



**Graph 2:** Mean scores of gradings squamous cell carcinoma according to groups

Likewise, there were two false-negative correlation obtained in squamous cell carcinoma group, with 13 positive correlations.

## DISCUSSION

The popularity of FNAC depends on the simplicity of the technique, rapidity of the report and low cost. Both as a preoperative investigative modality and postoperative monitoring procedure, FNAC carries little risk or stress to the patient. FNAC has been shown to be a reliable and safe technique in the diagnosis of malignancies in the head and neck. It has been employed extensively in the study of lymph nodes, salivary glands and bone lesions. However, very limited data are available with regard to FNAC of indurated oral mucosal lesions, though the mouth is readily accessible for this procedure.<sup>1</sup>

Due to the time-tested superior diagnostic accuracy of formal biopsy procedure, one is most likely to caution against the use of FNAC as a conclusive, routine primary diagnostic modality for indurated oral mucosal lesions. Moreover, the oral cavity is directly and easily accessible to formal biopsy procedure. Traditionally, biopsy has been a 'gold standard' for cytologic reliability. Yet, we know that biopsy has its own limitations and contraindications. The reliability of FNAC vs biopsy is deemed useful in assessing the possibility of employing the FNAC as a test for the diagnosis of oral squamous cell carcinoma, at least in medically compromised cases.<sup>9,8</sup>

FNAC was shown to be simple, safe and effective diagnostic procedure in studying gingival changes in patients with acute leukemia. Thus, in a patient reporting with gingival bleeding and/or gingival enlargement without commensurate presence of deposits and relevant history, acute leukemia could be suspected and the patient should

be referred for hematological opinion as the importance of this disease to the dentist cannot be overemphasized, since, he is in an ideal position to detect it relatively early in its course.

To exclude the possibility of contamination of the aspirated material by blood, each aspirated sample was compared with the peripheral blood and the following points were used for comparison differences in the degree of gingival infiltration and the white blood cells in the peripheral blood and the difference between the type of cells in the aspirate and that in the peripheral blood.<sup>12</sup>

Scrofula is a local manifestation of the systemic disease. It may occur during primary tuberculous infection or as a result of reactivation of dormant foci or direct extension of the bacilli to the hilar lymph nodes. A high index of suspicion is needed for the diagnosis of mycobacterial cervical lymphadenitis. A thorough history and physical examination, tuberculin test, staining for acid-fast bacilli, radiologic examination and FNAC will help to arrive at an early diagnosis of mycobacterial lymphadenitis which will allow early institution of treatment before a final diagnosis can be made by biopsy and culture. FNAC and tuberculin skin test helped in arriving at the diagnosis.<sup>13</sup>

The slides were wet fixed with 1:5 ether alcohol. Two slides were prepared for each patient. One slide was stained with H&E and another one slide with rapid PAP stain.<sup>1</sup> An incisional biopsy was performed at the site of FNAC. Formalin-fixed, paraffin-embedded specimens were then sectioned to 0.5 mm thickness and stained with routine H&E stain. FNAC and histopathologic reports of these patients were reviewed to find out the accuracy of FNAC with the HPE report.<sup>1,2,7,8</sup>

In the present study, the results of the dysplasia category are poor compared to the squamous cell carcinoma category. The superior result of squamous cell carcinoma is attributable to ease in obtaining the specimen by FNAC, which is tardy in leukoplakia. The increased number and reduced cohesiveness of the cells in squamous cell carcinoma may be responsible for the ease in specimen collection. Squamous cell carcinoma smears showed isolated cells or clusters of keratinizing malignant squamous cells with or without evidence of keratin formation; cells had distinct cell borders, hyperchromatic nucleus with coarse chromatin. Smears showed large pleomorphic cells with high nucleocytoplasmic ratio and prominent nucleoli. Binucleate and multinucleate forms were also seen. A well-defined cytoplasm, absent or few stripped nuclei and a bland finely granular nuclear chromatin scanty cytoplasm, a high N:C ratio, naked nuclei, nuclear molding, nuclear hyperchromasia and coarseness has been observed (Fig. 5).

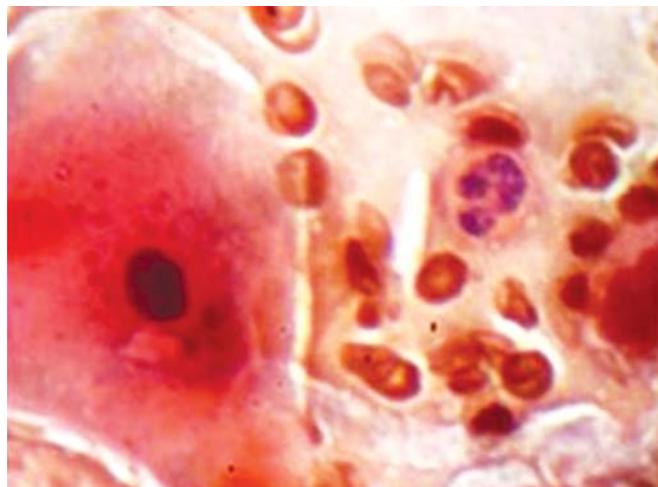


Fig. 5: Cytological feature of squamous cell carcinoma

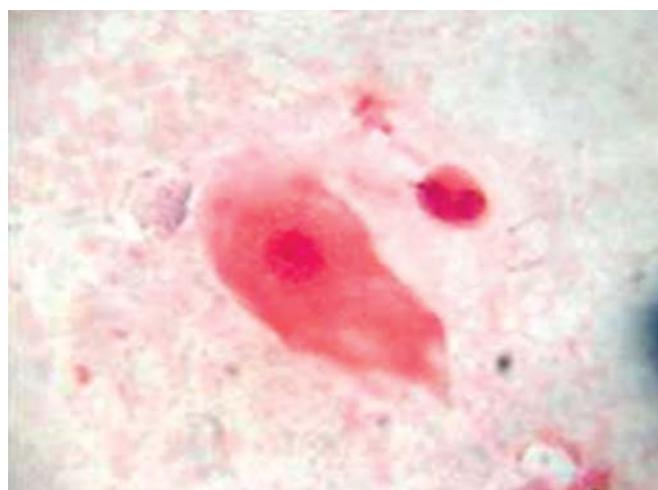


Fig. 6: Cytological feature of oral leukoplakia

‘One oral leukoplakia smear showed abundant cytoplasm, round nuclei, fine chromatin and inconspicuous nucleoli. Nuclear pleomorphism (Fig. 6).’

In the present study, 15 cases of oral squamous cell carcinomas and 15 cases of oral leukoplakia, we found that in leukoplakia, FNAC results was true positive in 6.6% and 93.4% was false negative, whereas in cases of oral squamous cell carcinoma, 86.6% were true positive and 13.4% were false negative.

In our study, it was found that in leukoplakia group sensitivity was found to be 93.33% and specificity being the same as sensitivity, in oral squamous cell carcinoma group the sensitivity was 0.0% and specificity was 100%.

When all the obtained FNAC reports were compared with histopathology reports, it was found that in 14 samples of leukoplakia group where FNAC showed negative results were turned out to positive histopathologically.

Likewise, there were two false-negative correlation obtained in squamous cell carcinoma group with 13 positive correlations.

## CONCLUSION

The study attempts to determine the possibility of using FNAC as a primary diagnostic test in oral leukoplakia and squamous cell carcinoma, evaluate the benefits and limitations of FNAC, evaluate the degree and causes of under diagnosis of incisional biopsy in oral leukoplakia and squamous cell carcinoma. The ability to control oral cancer and precancer will depend on two cornerstones: Prevention and early diagnosis.

Continuing educational campaigns are needed on the local, state and national level in order to educate the public about the risk factors and early signs/symptoms associated with this disease. Individuals also need to be encouraged to seek regular professional oral examinations by a dentist and/or physician. Finally, health care workers must be encouraged to perform oral cancer examinations as part of their patient care regime and to be knowledgeable about early signs of oral carcinoma.

Concluding from this study, it is noted that FNAC can be employed as a sound diagnostic tool for rapid diagnosis of oral squamous cell carcinoma.

In the developing third world countries, where a blade carries the surgical impression, whereas a needle enjoys a therapeutic image, the potential role of FNAC for diagnosis of oral squamous cell carcinoma is far reaching and, to a large extent, it is yet to be explored.

## CLINICAL SIGNIFICANCE

The role of FNAC in diagnosis of oral cancer is safe, simple, economic, well-tolerated, relatively painless and an ambulatory procedure. It also carries little risk or stress to the patient. FNAC has been shown to be reliable and safe technique in the diagnosis of malignant in the head and neck. When the aspirations are performed by cytopathologists, it is easy to perform a rapid staining of the first smear and within 10 to 15 minutes to ensure that the material is sufficient and diagnosable and to suggest a preliminary diagnosis.

## REFERENCES

1. Seetharam SS, Ramachandran CR. Fine needle aspiration cytology as a diagnostic test for oral squamous cell carcinoma. *Oral Diseases* 1998;4:180-86.
2. Akerman M. Fine needle aspiration cytology of soft tissue sarcoma: Benefits and limitations. *Sarcoma* 1998;2:155-61.
3. Singh S, Gupta S. Fine needle aspiration cytology in lesions of oral and maxillofacial region: Diagnostic pitfalls. *J Cylol* 2011;28(3):93-97.
4. Gandhi S, Lata J. Fine needle aspiration cytology: A diagnostic aid for oral lesions. *J Oral Maxillofac Surg* 2011 June;69(6):1668-77.
5. Elagoz S, Gulluoglu M, Yilmazbayhan D, Ozer H, Arslan I. The value of fine needle aspiration cytology in salivary gland lesions. *ORL J Otorhinolaryngol Relat Spec* 2007;69:51-56.
6. Singh D, Sinha BK, Shyami G, Baskota DK, Guragain RPS, Adhikari P. Efficacy of fine needle aspiration cytology in the diagnosis of oral and oropharyngeal tumors. *Int Arch Otorhinolaryngology* 2008;12:47-54.
7. Rhodus NL. Oral cancer: Leukoplakia and squamous cell carcinoma. *Dent Clin N Am* 2005;143-65.
8. Zbaren P, Nuyens M, Loosli H, Stauffer E. Diagnostic accuracy of fine needle aspiration cytology and frozen section in primary parotid carcinoma. *Cancer* 2004;100:1876-83.
9. Lu BJ, Zhu J, Gao L, Xie L, Xu JY, Lai MD. Diagnostic accuracy and pitfalls in fine needle aspiration cytology of salivary glands: A study of 113 cases. *Zhonghua Bind Li Xue Za Zhi* 2005;34:706-10.
10. Orell SR, Strett GE, Whitaker D. Introduction. In fine needle aspiration cytology. Elsevier Limited 2005;4:1-8.
11. Rajwansi A, Gupta K, Gupta N, Shukla R, Srinivasan R, Nijhawan R, Vasishta R. Fine needle aspiration cytology of salivary glands: Diagnostic pitfalls revisited. *Diagn Cytopathol* 2006;34:580-84.
12. Sri Kennath A, Arul J, Verma Sonika. A clinical and fine needle aspiration cytology study of gingiva in acute leukemia *Dent Res J (Isfahan)* Jan-Mar 2012;9(1):80-85.
13. Sudhakara R, Reddy K Ramya. Scrofula in young female—case report. *Int J Dent Case Reports* 2012;2(2):41-45.
14. Manyam R, Reena K, Saraswathi TR. Role of deeper sections in diagnostic oral histopathology: A retrospective study. *IJDR* 2011;22(1).
15. Singh Nanda, Kanwar Deep. Fine-needle aspiration cytology: A reliable tool in the diagnosis of salivary gland lesions. *J Oral Pathol Med* 2011;41(1).
16. Vaidya S, Sinha A. A comparative study of fine-needle aspiration cytology and histopathology in salivary gland lesions. *J Pathol Nepal* 2011;1:108-13.
17. Mistry RC, Quresh SS. Cervical lymph node metastases of squamous cell carcinoma from an unknown primary: Outcomes and patterns of failure. *Indian J Cancer* 2008;45(2):54-58.
18. Nagaraju K, Prasad S, Ashok. Diagnostic efficiency of toluidine blue with lugol's iodine in oral premalignant and malignant lesions. *Indian J Dent Res* 2010;21(2):218-23.

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