

Role Of Cytology In Oral Mucosa Leisions – A Study Of 80 Patients

Abstract

Current study describes cytological findings of scrape smears taken from patients of oral mucosa lesions presenting in clinical practice. The study was done in 80 patients of oral mucosa lesions who were enrolled over a period of 2 years at a tertiary care hospital. The study shows that mucosal diseases are more frequently seen in middle aged population. Similarly, these group of patients have reservations regarding invasive procedures like mucosal biopsy and thus if given a choice will opt for minimally-invasive diagnostic technique like mucosal scrape cytology. Scrape cytology is a rapid, simple and inexpensive diagnostic test which can be easily performed in day-to-day practice. This study is being conducted to practice and observe the changes in cell morphology in different diseases of the mucosa. Few of the advantages of using exfoliative cytology for diagnosing mucosal lesions are that all these techniques are simple, non invasive, cost effective and can be performed on those patients who are considered not fit for surgical procedures or refuse to undergo biopsy. Also cytology delivers a quick result thus treatment delay may be avoided. It is a simple and rapid mode for initial opinion of the lesions and may help in deciding the treatment plan which is likely to get delayed when a biopsy is done. The average time of a biopsy reporting is 3-4 days, whereas cytology report can be made available on the same day. It can also be used for patients who are considered not fit for surgical procedures

Key Words

Cytology, Oral, Leisions, Mucosa

Introduction

Oral Mucosal lesions are very common presentations in dental practice. The lesions which may involve Oral mucosa are Leukoplakia, Lichen Planus, Acute Ulcerative gingivitis, Syphilis, Candidiasis, Bullous Erythema Multiformae.

Most of these lesions are either whitish or reddish coloured plaques or erosions/ulcers with difficulty in diagnosing the cause on the basis of appearance alone. A thorough history and associated clinical features have to be considered along with investigations to reach a conclusion regarding the diagnosis. Among the investigations, The Gold standard is Mucosal biopsy. But one major disadvantage of mucosal biopsies is that it is an invasive procedure, with a slight delay in getting the results with conventional laboratory techniques. Thus to minimize trauma, infection and bleeding along with rapid bedside interpretation, various other methods of cytology are being tried. Few of the more common ones being - Exfoliative cytology, Touch imprint cytology, Liquid based cytology. Few of the advantages of using exfoliative cytology for diagnosing mucosal lesions are that all these techniques are simple, non invasive, cost effective and can be performed on those patients who are

considered not fit for surgical procedures or refuse to undergo biopsy. Also cytology delivers a quick result thus treatment delay may be avoided^[1]. Limitations include less and superficial tissue representation, distorted pathology and poor reliability in samples taken from vermilion borders of lips.

This study is being conducted to practice and observe the changes in cell morphology in different oral diseases of the mucosa.

Aims & Objective

To study oral mucosa cytology for the diagnosis of mucosal lesions in patients presenting as out-patient in clinical practice.

Material and Methods

This study was conducted at tertiary care hospital. The study protocol, patient information sheet and consent form were approved by the institutional ethics committee. Written informed consent was obtained from all patients.

A cross sectional study included patients with mucosal lesions attending out-patients department (OPD) at tertiary care hospital and referral centre. A total of 80 patients satisfying inclusion and exclusion criteria were included in the

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study.

Inclusion criteria

- Patients having oral mucosal lesions attending out-patient department or referred from ENT OPD, skin OPD irrespective of age or sex.
- Patients willing to participate in the study.

Results

A study of 80 patients of oral mucosal lesions was done. Duration of the study was two years.

The following observations and results were drawn.

Age Distribution:

The age of patients varied from 18 years to 70 years. The mean age of patients in our study was 37.35 ± 12.3 SD years. Most of the patients belonged to 3rd and 4th decade of life.

Sex Distribution:

Out of 80 patients 52 were male and 28 were female. The sex distribution can be seen in **Table 1** below.

Disease Distribution:

Maximum numbers of patients were of Pemphigus Vulgaris (27%), followed by patients with drug reactions (24%) and

Table 1: Sex Distribution Of Patients

Sex	No Of Patients
Males	52
Females	28
Total	80

Table 2: Disease Distribution In Patients

Disease	No. Of Patients	Percentage
Pemphigus Vulgaris	23	28.75%
Drug Reactions	16	20%
Herpes Simplex	13	16.25%
Apthous Ulcer	8	10%
Carcinoma	6	7.5%
Oral Lichen Planus	6	7.5%
Erythroplakia	2	2.5%
Leukoplakia	2	2.5%
Oral Candidiasis	2	2.5%
Traumatic Erosion	2	2.5%

Herpes Simplex infection (16%). The disease distribution is shown in **Table 2**.

Cytopathology

1) Pemphigus Vulgaris

Acantholytic cells were the most common findings in pemphigus patient's mucosal scrape cytology. Clear large round nucleus was well appreciated whereas cytoplasmic extensions were not seen. The inflammatory cells were predominantly neutrophils.

2) Herpes Simplex Infection

There was nucleomegaly in all patients, with the nucleus size 3-5 times that of normal. The inflammatory infiltrate was predominantly neutrophilic in all the patients, with eosinophils being the second most common inflammatory cells seen. Presence of bacteria in 6 patients suggested secondary bacterial infection.

3) Candidiasis

Fungal spores and Pseudo-hyphae were seen in all patients, whereas presence of yeast cells was seen in only 2 patients. The inflammation was very severe in all patients with predominant cells being Neutrophils.^[2]

4) Drug reactions

The most common finding in mucosal cytology of drug reaction group was nuclear enlargement. Pyknosis and Chromatin condensation were the next most common observations. Inflammation was severe in most patients. Neutrophils were predominant inflammatory cells seen. 2 patients had presence of intracytoplasmic melanin in the cytology slides.

5) Aphthous Ulcers

Nucleomegaly and presence of chromocentres were the most common findings seen in all patients. The inflammation ranged from moderate to severe, with Neutrophils and Eosinophils being the more common inflammatory cells.

6) Carcinoma

Keratinised pearls, Nuclear enlargement and Orange - yellow stained cytoplasm were seen in 4/6 patients. Pyknosis was seen in only 2 patients. Ghost cells were not seen in any of the 6 patients.

7) Leukoplakia

The cytology of Leukoplakia showed presence of marked nucleomegaly in the 2 patients under this study. Chromocentre was seen. Moderate inflammation with presence of neutrophils and lymphocytes was seen. No plasma cells were seen.

8) Oral Erosive Lichen Planus

The cytology of oral erosive lichen planus showed presence of Mature squamous cells in 6/6 patients. Mild nucleomegaly was seen in 4/6 patients. Reactive nuclear atypia was seen in 4/6 patients. Dark orange cytoplasm was seen in 2/6 patients. Moderate Neutrophilic inflammation was seen 6/6 patients. .

9) Erythroplakia

Scantly cellularity was seen with very few scattered neutrophils. No opinion was possible.

10) Traumatic Erosion

The cytology of traumatic ulcer showed moderate neutrophilic inflammation in the two patients under this study. The squamous cells were of normal size, with normal sized clear round nucleus. There was presence of multiple bacteria, suggestive of secondary bacterial infection.

Discussion

Current study describes cytological findings of scrape smears taken from patients of oral mucosa lesions presenting in clinical practice. The study was done in 80 patients of mucosal lesions who were enrolled over a period of 2 years at a tertiary care hospital.

The mean age of patients in our study was 37.35 ± 12.3 SD years. Age range varied from 18 to 70 years. Maximam patients belonged to 3rd and 4th decade of life indicating that mucosal diseases are more frequently seen in patients in middle aged

population. Similarly, these group of patients have reservations regarding invasive procedures like mucosal biopsy and thus if given a choice will opt for minimally-invasive diagnostic technique like mucosal scrape cytology.

In our study, mucosal lesions were present in 52 males and 28 females. The higher prevalence of mucosal lesions in males seen in our study could be due to more awareness and reporting to the physician by males. The low percentage of female patients with lesions in our study could be attributed to various factors like shyness and apprehension, which might lead to under-reporting in female patients.

In our study, we used wooden tongue depressor/ spatula for scraping of the lesions. In majority of the studies on scrape cytology, Cytobrush or Ayre's spatula is used. This is done because of the fact that Cytobrush, having bristles all around, can take better samples just by rolling it over the lesion. The Ayra's spatula can easily negotiate body contour of orificial mucosa like vagina or mouth. Green M.et al actually compared utility of cytobrush was more efficient in sampling of mucosal lesions as compared to wooden spatula^[2]. Cell yield of the smears was better when cytobrush was used. However, we used a wooden spatula in our study as it is readily available in day to day practice and is relatively inexpensive.

After scraping, we used 95% ethanol as the fixative for all cytology slides. This was done as 95% ethanol is commonly used as fixative for cytology slides worldwide. This is also done because all cytology slides are stained within 15 minutes of collecting samples.^[3]

A minimum of 2 slides were prepared for each patient, and while staining, one of the slides was stained by the Papanicolaou (PAP) staining procedure and the other by Haematoxylin and Eosin (H & E) staining procedure. Both staining methods were used so that nuclear and cytoplasmic details are better appreciated. This is comparable to study done by Janete Dias Almeida et al who compared four different staining methods for mucosal cytological diagnosis^[4]. In our study, better cellularity and nuclear details were appreciated by Papanicolaou staining of the smear. This is consistent with the findings of Janete Dias Almeida et al, who found Papanicolaou staining to be better as compared to Haematoxylin and Eosin for staining of mucosal smears.

Pemphigus Vulgaris

Out of total 80 patients, pemphigus vulgaris was most commonly observed mucosal disease. This number is significantly more than the study of 9 patients of pemphigus vulgaris by Jarkko Hietanen et al and the study of 9 patients of genital pemphigus vulgaris by Fairbanks Barbosa ND et al^{[15],[6]}. In these studies, cytologic details of acantholytic cells are not described. However, most of them have used Tzanck stain of Leishman instead of Pap stain and therefore could not describe finer morphologic details^{[7],[18]}

The most common findings in pemphigus vulgaris patients in our study was the presence of acantholytic cells. This was comparable to the findings of Sabir F et al, who found acantholytic cells in 9/12 (75.00%) patients of pemphigus vulgaris^[9]. Medak et al found acantholytic cells in oral mucosal smears of 9/9 (100%) patients of pemphigus vulgaris. Similarly, Fairbanks Brabosa ND et al found acantholytic cells in genital scrape smears of all 9 (100%) patients of pemphigus vulgaris in their study^[6]. Moderate to severe inflammation surrounding acantholytic keratinocytes was seen in all slides.

Drug Reaction

The other major group in our study was drug reactions with mucosal lesions. Cytology findings in only a single patient of oral ulceration secondary to antidepressant drug have been described by Fernanda Bertini et al. They reported the presence of necrotic cell, however description of detailed morphology of necrotic cells was missing.^[10]

The most common findings in drug reaction patients in our study was the presence of enlarged nucleus in cells. Other features seen were pyknotic nuclei, cell membrane damage and cytoplasmic vacuolization. These are features of necrotic cells.^[11]

In our study, nuclear enlargement was also found in patients of aphthous ulcers, pemphigus, herpes infection and malignancy. However, nucleomegaly in these patients was marked and much more than that of drug reaction patients.

Herpes Simplex Infection

The third group of patients in our study was that of herpes simplex infection patients. Kobayashi et al has studied 11 patients of herpes simplex infection while Mary R. Motyl et al reported cytologic findings in 21 patients of

herpes infection.

The most common findings in herpes simplex infection in our study was the presence of 'Ground Glass' nucleus and chromatin condensation. These findings can be explained by invasion of the nucleus by herpes virus leading to condensation of the chromatin at periphery of the nucleus and 'ground glass' appearance of the nucleus. The virus also causes nuclear multiplication, resulting in crowding and multinucleation. The few number of patients with multinucleated giant cells could not be explained, but may be attribute to early stages of the disease, where virus induced nuclear multiplication has not yet begun. The nucleus was enlarged 3-4 times that of normal in all the patients.

Aphthous Ulcer

The fourth group comprised of patients with aphthous ulcers which is less when compared to the study of Banoczy J et al done on 72 patients with recurrent aphthae.^[13] This low number of patients can be attributed to the fact that ours is a tertiary care referral centre and most of the patients of aphthous ulcers are treated before coming to a tertiary care hospital.

The aphthous ulcer patients showed enlarged nucleus and presence of chromocentres. There was presence of mononuclear macrophages. These findings are suggestive of inflammatory disease, as nuclear enlargement and chromocentres may be seen in acute inflammatory diseases, and are not specific for aphthous ulcers as such.

Number of patients with aphthous ulcers in our study was limited. More studies are required to confirm our cytologic observations in aphthous ulcers.

Carcinoma

The patients of malignant ulcers comprise the next group. This number is significantly lesser than the various cytology studies on oral malignant conditions.

The carcinoma patients showed presence of keratinized pearls, nuclear enlargement and orange yellow stained cytoplasm. These findings are consistent than those reported by Divani S et al. who found keratin pearls and nuclear enlargement in 4/36 (11.11%) patients.^{[14],[15]}

Oral Lichen Planus

There were 6 (7.5%) patients of oral lichen planus in our study. P B Sugerma et al studied 10 oral lichen planus

patients.^[16] But they only considered the cytoplasmic/nuclear ratio of scrape smears of lesional mucosa, which they found was reduced as cytoplasmic area was reduced in the cells. Also, they found that keratinisation was increased in oral lichen planus. But, comment on morphological details of the cells was missing.

In our study, there was presence of mature normal sized squamous cells in 6 patients (100%). Mild nucleomegaly was noted in 4 patients along with reactive nuclear atypia.

Other Diseases

Other patients included in the study were leukoplakia, erythroplakia, and traumatic erosion. We could not trace any significant and important studies with detailed cytological analysis for these conditions during the course of our study.

Advantages Of Cytology For Mucosal Lesions

The advantages of scrape cytology of mucosal lesions are manifold. It is a simple and rapid mode for initial opinion of the lesions and may help in deciding the treatment plan which is likely to get delayed when a biopsy is done. The average time of a biopsy reporting is 3-4 days, whereas cytology report can be made available on the same day. It can also be used for patients who are considered not fit for surgical procedures. A number of patients refuse invasive procedure like biopsy on oral or genital areas. Skin biopsy, although the gold standard can not be performed in every case. Cytology can be used for a mucosal lesion that appears clinically innocuous and otherwise would not be biopsied. This procedure is minimally invasive, and thus less traumatic and less painful for the patient. If adequately trained, the procedure is easy to perform in OPD can be done with minimal equipments. The cost of investigation is less as compared to biopsy. It can assist physician in taking decisions with regard to treatment. Similarly, anxious queries of patients regarding whether a lesion is malignant or not can be partially answered by way of cytology.

Disadvantages Of Cytology For Mucosal Lesions

There are few disadvantages of mucosal cytology, as assessed by our study. Considerable skills are required for preparation of good smears. Being a cytology technique, availability of considerable cellularity in scrape smears

is of paramount importance. However this drawback can be overcome to some extent by doing initial toluidine blue staining. The stage of the disease and the timing of smear preparation may alter the findings of the smears. However this holds true even for mucosal biopsy or other investigations. Fixatives have to be kept available, as unfixed smears will not give accurate findings. Expertise in interpretation of the slides is limited as there is lack of previous studies on scrape cytology in inflammatory diseases. By this procedure, we are unable to distinguish between various inflammatory diseases like Behcet's disease, traumatic erosion, contact dermatitis, aphthous ulcers etc. Scrape cytology may not be useful for diagnosis of mucosal malignancies and tissue biopsy thus remains the gold standard and should be recommended in every case of suspected malignancies and tissue biopsy thus remains the gold standard and should be recommended in every case of suspected malignancy.

As cytology is a simple office procedure, it should be done in as many patients of mucosal lesions as possible, so that more data is collected and better expertise is achieved for interpretation of cytological findings.

Our study has some limitations. Cytobrush could have been used. Histopathologic correlation was not done in all cases and was done only in few cases where diagnosis of mucosal diseases was not established based on clinical examination alone. Cytologic and histo-pathological correlation should be done in maximum possible patients, so that there can be more accurate analysis of cytology smears.

Summary & Conclusions

1. Current study describes cytological findings of scrape smears taken from patients of mucosal lesions presenting to out patient department. The study was done in 110 patients of mucosal lesions who are enrolled over a period of 2 years at a tertiary care hospital.
2. Scrape cytology is a rapid, simple and inexpensive diagnostic test which can be easily performed in day-to-day practice.
3. Cytology can be done for patients who are apprehensive, or do not consent for a mucosal biopsy.
4. Adequate cellularity of keratinocytes is essential for interpretation of cytology smears.
5. Of the various staining methods,

Papanicolaou staining allows better visualization of nuclear and cytoplasmic details on light microscopy followed by Hematoxylin and Eosin staining.

6. Cytologic findings are characteristic for the diagnosis of mucosal pemphigus vulgaris, herpes simplex infection and mucosal candidiasis.
7. A large homogenous clear nucleus with thin rim of cytoplasm was the most consistent cytologic finding in pemphigus vulgaris. 'Ground glass' nucleus and chromatin condensation was the consistent and specific cytologic finding in herpes infections.
8. Mild to moderate nucleomegaly was the main cytologic observation in majority cases of drug reaction, aphthous ulcers and lichen planus can be considered as a sign of cell injury due to inflammatory process.
9. Inflammatory cells in the background offers valuable clue for the diagnosis of certain diseases like Aphthous ulcers in which dense neutrophilic infiltrate is observed.
10. Instead of multiple investigations like Tzanck smear, KOH mount, Gram's stain, a single investigation i.e. Scrape smear with Papanicolaou staining, can be utilized effectively to differentiate between various mucosal diseases commonly presenting to dermatology outpatient.

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