The Value of Cytobrush Technique in The Diagnosis of Oral Ulcerative Lesions

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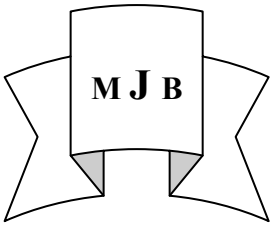

Abstract

A total of 49 patients were included in this study, 29 patients with oral benign and malignant lesions, and 20 healthy individuals were included as control.
All these cases were examined by using cytobrush technique.
The study aimed to evaluate the sensitivity and specificity of cytobrush method in the detection of precancerous and cancerous lesions of oral mucosa.
The results of cytobrush method were compared with those of scalpel biopsy of suspicious oral lesions.
The brush method has high sensitivity and specificity rate (88% sensitivity, 100% specificity). The cytobrush method was a highly accurate method of detecting oral precancerous andcancerous lesions (93% accuracy rate).
Although the histological pattern was the definitive diagnosis in those patients, but when the lesion was doubtful, the cytological examination (cytobrush technique) was not only supportive but also diagnostic. It can aid in confirming the nature of apparently benign oral lesionsand, more significantly, revealing those that are precancerous and cancerous when they are not clinically suspected of being so.
All cytobrush positive results should be referred for scalpel biopsy and histology to completely characterize the lesion.

الخلاصة

شملت هذه الدراسة ٤٩ شخصا، تسعة وعشرون منهم يعانون من تقرحات فموية حميدة أو خبيثة، أما العشرون الآخرون فهم أصحاء وتم اعتبارهم كمجموعة سيطرة. كل هذه الحالات تم فحصها باستعمال طريقة الفرشاة الخلوية.

الهدف من الدراسة هو تقييم حساسية وخصوصية طريقة الفرشاة الخلوية في تحديد التقرحات السرطانية وما قبل السرطانية التي تصيب بطانة الفم، ونتائج هذه الطريقة قورنت مع الطريقة المشرطية لأخذ العينة من التقرحات الفموية المتوقعة.

طريقة البابانيكولا استعملت لغرض صبغ المسحات التي أخذت بطريقة الفرشاة الخلوية، ويتم معاينتها تحت المجهر الضوئي.

أظهرت نتائج البحث أن هناك معدل عالي لحساسية وخصوصية هذه الطريقة (٨٨% للحساسية و ١٠٠% للخصوصية) ، لذلك فان طريقة الفرشاة الخلوية ذات دقة عالية في تعيين التقرحات الفموية السرطانية وما قبل السرطانية (٩٣% معدل الدقة).

نستنتج من ذلك انه بالرغم من ان الحالة النسجية تعطي التشخيص النهائي للحالة، إلا أن طريقة الفرشاة الخلوية ليست فقط داعمة للتشخيص بل هي طريقة تشخيصية أيضا.

Introduction

Detection of oral cancer in the early asymptomatic stage dramatically improves cure rates of patients, quality of life by minimizing extensive debilitating treatment.[1]

Unfortunately, most of patients with oral cancer display evidence of spread to regional lymph nodes and metastasis at time of diagnosis.[1]

Although screening has been emphasized as a method of reducing the morbidity and mortality associated with oral

cancers, the visual detection of oral cancer at an early stage is significantly hindered by the difficulty in clinically differentiating premalignant and malignant lesions from similar looking benign. An accurate, reliable method for diagnosing oral mucosal abnormalities has been remains the scalpel biopsy.[4]
However, when presented with the need to have an oral mucosal biopsy performed, the patient is often reluctant, and at times fearful, of such an invasive surgical procedure. The patients' reluctance may be compounded by the clinicians' hesitation to perform a surgical procedure in an unfamiliar setting or anatomical site. [2]
The use of cytobrush has a wide clinical application in assessment of surface oral and oropharyngeal mucosal abnormalities, which include leukoplakia and mixed red and white lesions, such as erythroplakia and speckled leukoplakia .
Early assessment of such lesions leading to prompt identification of dysplastic preinvasive or early minimally invasive disease is central to eliminating these lesions. This permits high cure rates even in cases where invasive disease is noted on subsequent scalpel biopsy.
The development of cytobrush method has brought accurate diagnosis, ease of performance, and patient acceptance into daily practice.
In contrast to traditional exfoliative cytology, the cytobrush, using a specially designed circular bristle brush, is able to access and sample all epithelial layers, including the basal layer and the most superficial aspects of lamina propria. Thus the cellular material obtained is a true representation of all epithelial layer in a disaggregated form spread over the surface of an ordinary glass slide .[2]

Aims of the study

The present study aims at
1- Detecting of morphologic and cellular abnormalities of suspicious oral lesions using cytobrush technique.
2- Comparing the results with those of scalpel biopsies.
3- Evaluating the specificity and sensitivity of cytobrush method in early detection of precancerous and cancerous oral ulcerative lesions, specifically, to determine the positive predictive value of an abnormal cytobrush.

Patients and Method

Subjects

The study was performed on 49 subjects and lasted for six months.
The patients were attending in Outclinic of Department of Maxillofacial surgery of Specialized Surgeries Hospital in Baghdad.
29 patients having oral ulcerative lesions that had an epithelial abnormality of undetermined etiology, all patients were older than 18 years. Patients with oral lesions covered with clinically intact normal epithelium such as mucoceles, fibroma and pigmented lesions were included in this study.
The requisition form included demographic data such as the patients age, sex and history of smoking and alcohol intake, as well as the location and clinical description of the oral lesions, including the predominant color, morphology, sign and symptoms.
As control, oral smears were obtained from 20 individual with apparently normal mucosa.

Preparation of Smears

Cytobrush technique:

This technique uses a round stiff bristle brush to collect cells from surface and sub-surface layers of a lesion by vigorous abrasion .The brush is rotated in one spot until bleeding starts, to ensure a sufficiently deep sample.
Pinkness of tissue or pinpoint bleeding at the brush cytology site was evidence of proper technique [2]. Neither topical nor local anesthetic was used. Modified brush (interdental brush) was used in different size according to demand.(jourdan type), it resembles the

endocervical brush which is used for cervical cytololgy, there is no additional tools or devices were used with this method.

Fixation of smears

The cellular material collected on the brush was transferred to the coded glass slide and rapidly flooded with the fixative to avoid air drying.

Two fixed slides were taking for each case.

The main purposes of cytologic fixative are to penetrate cells rapidly, minimize cell shrinkage and maintain morphologic integrity.

The fixative used is 95% ethanol. Immediate fixation while the specimen is still wet, is essential for preservation, as, even minimal air - drying of sample will alter cellular features.

The minimal fixation time is 15 minutes, but prolonged fixation of several days will not alter the appearance of the smear.

Staining Method

The Papanicolaou method was used for staining [9].

The staining procedure was devised for optimal visualization of cancer cells exfoliated from epithelial surfaces of the body.[3]

The specimens were classified into one of the following four categories:-

1- Negative: no epithelial abnormality.

2- Atypical: abnormal epithelial changes of uncertain diagnostic significance.

3- Positive: definitive cellular evidence of epithelial dysplasia or carcinoma.

4- Inadequate: scanty cellular elements.

Statistical Analysis

The result were presented by the use of SPSS and analyzed by chi square with probability less than 0.05 to be significant.

The following items were measured after comparing the result of reports of histopathological with the findings of cytological technique:-

1- Sensitivity: a measure of the like hood that a patient with dysplasia or carcinoma will have abnormal brush biopsy results, express as percent.

2- Specificity:- a measure of the like hood that a patient with a lesion determined to be benign by histology will not have an abnormal brush biopsy results and express as well in percent.

$$\text{Sensitivity} = \frac{\text{True + ve}}{\text{(False – ve) + (True + ve)}} \times 100$$

$$\text{Specificity} = \frac{\text{True - ve}}{\text{(False + ve) + (True - ve)}} \times 100$$

3- Positive predictive value: The probability that an oral lesion with an abnormal oral cytobrush results will prove to be precancerous or cancerous on scalpel biopsy.

4- Negative predictive value: The probability that an oral lesion without an abnormal cytobrush results will prove to be benign or –ve on scalpel biopsy.

$$\text{Predictive value of +ve results} = \frac{\text{True +ve}}{\text{(True +ve) + (False +ve)}} \times 100$$

$$\text{Predictive value of -ve results} = \frac{\text{True –ve}}{\text{(True –ve) + (False –ve)}} \times 100$$

$$\text{Accuracy} = \frac{(\text{True+ve}) + (\text{True –ve})}{(\text{True -ve}) + (\text{False -ve})} \times 100$$

Total

True positive: brush biopsy–a typical epithelial cells ; scalpel biopsy– epithelial
dysplasia or carcinoma present.

5- True negative: brush cytology– negative for epithelial abnormality; scalpel biopsy– no epithelial dysplasia or carcinoma present.

6- False positive: brush cytology–a typical cells; scalpel biopsy– no epithelial dysplasia or carcinoma present.

7- False negative: brush cytology– negative for epithelial abnormality; scalpel biopsy–squamous cell carcinoma present.

Results

AGE and SEX distribution

The result revealed that in 29 patients with oral ulcerative lesion, all of them were examined by scalpel biopsy and cytobrush 16 was malignant and 13 was benign (by scalpel biopsy method) and14 malignant , 15 benign (by cytobrush method).

Their age range from 18 years to >75 years.

The results of scalpel biopsy method were as follows 16 (55.2%) have positive result or malignant lesion and 13 (44.8%) have negative result or benign lesion.

While cytobrush result were: 14(48.3%) from the patients have +ve or malignant lesion and 15(51.7%) have benign lesion (-ve result).

So only 2(12.5%) result that were –ve in cytobrush method were +ve in scalpel biopsy which mean these 2 results were false –ve.

Specificity and sensitivity:

Table 1 Scalpel biopsy results and cytobrush result cross tabulation

		Scalpel Biopsy		
		+ve	-ve	total
Cytobrush	+ve	14	0	14
	-ve	2	13	15
	Total	16	13	29

According to this table:

*The result revealed that the sensitivityrate was 88%.
*The specificity rate was 100%.
*Predictive value of +ve result was 100%.
*Predictive value of –ve result was 87%.
*accuracy was 93%.

Cytopathological features

Smears of normal squamous epithelial cells show numerous, superficial and intermediate, polygonal, squamous cells which are shed singly or in large sheets. They have an abundant cytoplasm that is uniformly stained pink to blue, with a small, central, vesicular nucleus. As shown in fig.(1)

Various amounts of different inflammatory cells may also be seen in normal mucosa with gingivitis, which indicate an inflammation. As shown in fig (2)

The smear of leukoplakia lesion show an increased number of a nucleated cells with glassy-appearing, dens, orangeo-philic, abundant, hyperkeratotic cytoplasm.

They are differentiated from the normal a nucleated squamous cells of buccal mucosa by the density of their cytoplasm which results from the increased cytoplasmic keratins. As show in fig (3).

The principal features of squamous cell in benign disease of oral mucosa are relatively large, occasionally multiple , round or oval vesicular nuclei, small nucleoi may be noted . The cytoplasm is often poorly preserved. Leukocyte of various types are a common of smears in these finding. (fig. 4).

Cytologic smear from erosive lichen planus show intermediate squamous cell with large number of lymphocyte,(fig 5)

The smear of poorly differentiated sq cell carcinoma show squamous cell sheds singly, in clusters or in sheets, they have scanty to adequate non-keratinized cytoplasm and are evenly stained deep blue – purple.

In well differentiated sq cell carcinoma the cells are shed singly or in sheets with marked variation in size and shape (polygonal, spindle, pearl formation). Their nuclei always irregular, can be pyknotic or vesicular .

The chromatin is irregular clumped with pointed projection. The cytoplasm of the cells is thick and orageophilic and occasional keratohyalinic granular precipitate forming perinuclear rings . A nucleated, heavily keratinized (ghost like) cells are common in very well differentiated squamous carcinoma and may be the main cellular component in the smear. As shown in fig (6and7).

Discussion

The most effective method of combating oral cancer is early detection, diagnosis and eradication of early stage lesions and their precursors. The result of oral cytobrush method suggests that by bridging the gap between clinical inspection and histologic evaluation of oral lesions with epithelial abnormalities. The cytobrush could become instrumental in achieving this goal.

Since the oral cavity is the only region of the aerodigestive tract that can be effective screened, dentist should continue to be encouraged to perform oral cancer examination of all patients.

The most definitive accurate and reliable method for diagnosing oral mucosal lesion is the scalpel biopsy. The oral cytobrush has been developed as a technique for evaluating un explained clinically detectable alteration of the surface epithelium of the oral mucosa whether cancer or precancer is suspected.[3]

The goal of the oral cytobrush is to provide a highly sensitive and specific technique that is less painful and simpler to perform a scalpel or punch biopsy.

It considered as experimental and investigational for screening or diagnosis of cancerous or precancerous oral lesions.

It should be emphasized that oral cytobrush does not substitute for a scalpel biopsy; rather, it identifies oral lesions that require histologic evaluation.

When this technique detects cellular morphologic abnormalities, histology is necessary to further assess the architecture of the lesion. Therefore all atypical and positive result of cytobrush

should immediately indicate the need for scalpel biopsy and histologic evaluation, to completely characterize (that is to assign a stage and grade to the lesion).[1]

In many ways, the tool is analogous to the Papanicolaou (pap) smear for cervical cancer.

Although the pap smear is a screening test of a population without disease and the cytobrush tool is an adjunct diagnostic test for visible clinical abnormalities, both are simple , relatively inexpensive, risk free methods of screening for cancer and both serve as aids to the clinical examination. Just as visual inspection of the uterine cervix has shown to be an unreliable means of identifying precancerous and cancer, clinical inspection of the oral cavity has been shown to be equally unreliable precursor lesions and early cancers.[4, 5]

In this study, the cytobrush method proved to be adjunct to the oral examination in identifying precancerous oral lesion.

This method or test allows general dentists to determine the significance of epithelial abnormalities detected during routine oral examination.

Thus, the method aids in determining which benign looking lesions require immediate incisional biopsy.

In present study the cytobrush had 88% sensitivity and 6.98% false negative rate, in contrast to Sciubba s et al (1999) [1] study the sensitivity was 100% and zero false negative rate. The false negative rate in this study related to the presence of two results in cytobrush as a benign or (-ve) but is scalpel biopsy they were malignant (+ve). This error in cytobrush result occur due to incomplete access of the brush and sample all epithelial layers, including the basal cell layer and the most superficial aspects of the lamina propria . [3]

In this study the positive predictive value of an abnormal cytobrush was 100% while the study by Svirsky [6] it was 38%, this study suffers from the same weakness in that it does not adequately define the negative predictive value of the oral cytobrush results were followed by scalpel biopsy.

The study reported by Christian [7] suffers from the same weakness as only some of the positive oral cytobrush results and non of the negative results were followed by scalpel biopsy.

The present study disagree with Potter [8] study which conclude that the cytobrush technique may not be adequate sensitivity to detect all clinically dysplastic or malignant lesions, the sensitivity in present study was 100%. In addition Potter [8] criticize oral cytobrush as it adding time and cost to the diagnosis of oral lesions without additional benefit to the patient.

Actually cytobrush proved by many studies in addition to this study to be very accurate and beneficial method in diagnosis oral cancer in its early stage, especially for innocuous looking lesion, as precancerous and early stage oral cancers cannot be adequately identified by visual inspection alone and easily may be overlooked and neglected, even by trained professional with broad experience [5]. There are no previous Iraqi studies about using cytobrush technique in diagnosis of cancer and precancer oral lesions.

High rates of sensitivity and specificity achieved with this method reflects its accuracy and activity.

The oral cytobrush technique has been prompted as easier to perform than scalpel biopsy, such that dentists who are unskilled at perfoming scalpel biopsy may be able to perform oral cytobrush.[3]

Obviously, immediate biopsy of every oral lesion is impractical and not indicated when other simple, reliable, and acceptable technique are available to support clinical judgment in differentiating benign lesions from early

malignant changes, cytologic study is one such technique. [9]
Beside that direct smears allow for accurate sampling of a lesion and aid in complete screening.
Numerous reports substantiate the effect that use of oral cytology has accelerated biopsy of lesions that did not clinically appear to be oral cancer , thus leading to the early diagnosis of malignancies that would otherwise have remained temporarily unsuspected.[10-12]
Cytologic smears indicate the presence of suspicious or malignant appearing cells in most oral malignancies and useful in aiding clinical assessment of malignancies and accelerating biopsy. In exfoliative cytology most false- negative are derived from tissue that has a marked hyperkeratotic component. [9]
This reflects the difficulty of assessing cells as well as difficulties in sampling. While by using cytobrush instrument a full transepithelial biopsy specimen is obtained with minimal or no discomfort. As dysplastic and cancerous oral lesions frequently have an overlying keratin layer , cellular abnormalities in the deep basal layer of the epithelium are best sampled with this instrument .[1]
From clinical perspective, the white or leukoplakia lesion or such lesions with an erythematous component (erthroplakia) have a widely variable presenting appearance, are often asymptomatic and may appear innocuous.
Furthermore more worrisome- appearing lesions may ultimately exhibit benign, reactive, or inflammatory cellular abnormalities when a biopsy is performed. Such lesions are in a dynamic state, changing appearance in a relatively rapid time frame [13], therefore clinicians are not always able to accurately characterize or predict the behavior of such alteration based on the clinical characteristic alone.
One manner of sampling the broad array of white and red –white oral lesions is by way of the cytobrush technique. This efficient and readily accepted from of tissue biopsy allow the clinician access to the full thickness of the involved epithelial layer.[2]
The cytobrush is intended to evaluate benign – appearing oral lesions and not those distinguish by signs and symptoms of malignancies, which are clear signals for immediate incisional biopsy.
Patients with abnormal cytobrush results are more likely to be compliant with their dentists recommendations for scalpel biopsy and histologic evaluation than are those whose lesions have been identified by clinical examination alone. [14]

In conclusion, the result of this study remarks that oral cytobrush tool might enhance the oral cancer screening process by determining the significance and potentially harmful nature of identified oral lesions.
The cytobrush technique is not difficult to learn, so it can be integrated easily into the routine practice of dentistry.
The prospect of early oral cancer and pre-cancer detection that will result in prolonged survivor can offer patients and dentists hope?
In addition, the results demonstrate the potential value of cytobrush as an adjunct to the oral cavity examination in identifying precancerous and cancerous lesions at early stage.
The scalpel biopsy remaining the standard for arriving a definitive diagnosis, but use of cytobrush offers the clinicians a tool to help analyze surface oral mucosal abnormalities in a very scientifically based fashion.

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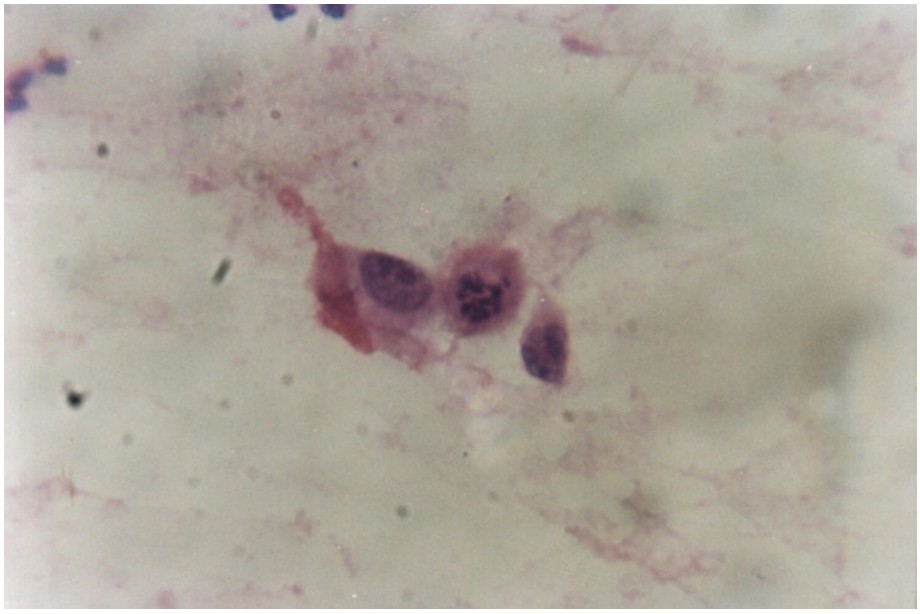

Figure1 The smear show 3 malignant cells showing course chromatin, with irregular nuclear membrane and keratinized cytoplasm. (keratinized SCC X400).

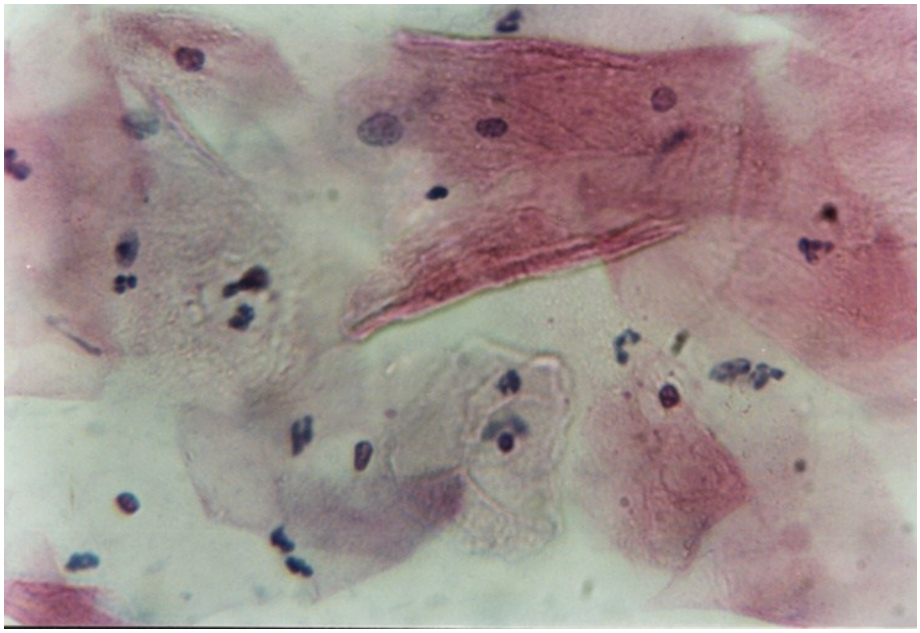

Figure 2 Smear show squamous cells with keratinized cytoplasm and moderate number of inflammatory cells.(leukoplakia X400).

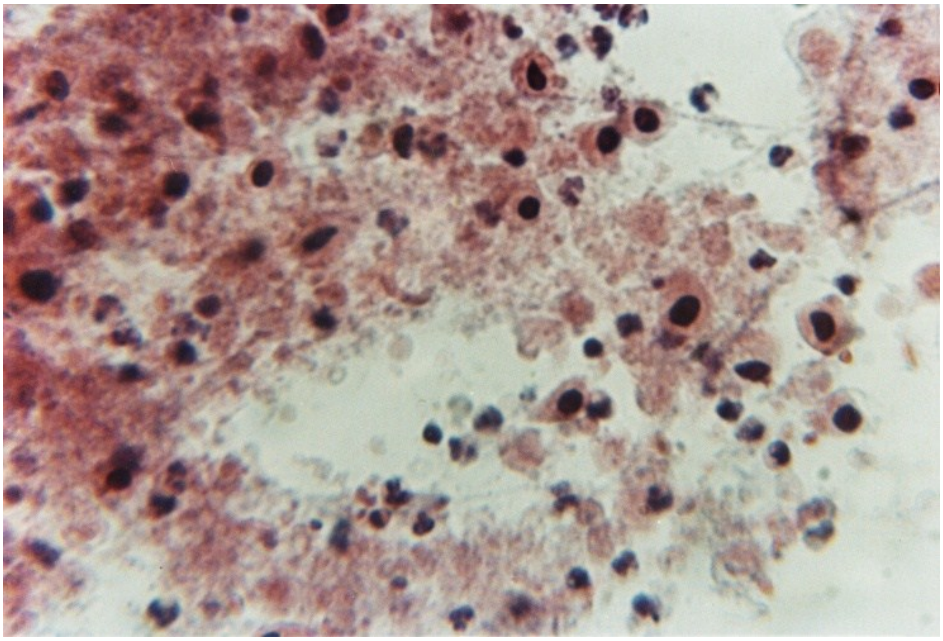

Figure 3 The smear shows sheets of malignant cells with malignant nuclei and keratinized cytoplasm.(keratinized SCC X400).

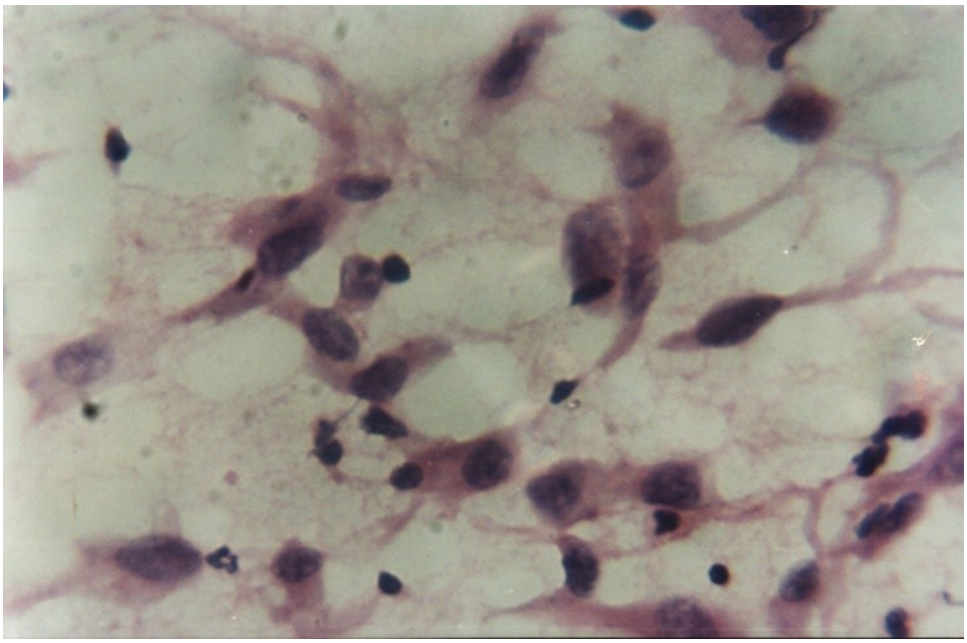

Figure 4 The smear shows cluster of malignant cells of different size and shapes ,showing malignant nuclei with course irregular chromatin and irregular nuclear membrane .(keratinized SCC X400).