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Original Article

Flourescent Staining in Liquid Based and Conventional Cytology: A Comparative Study

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ABSTRACT:

Background: Cancer is a major public health problem in modern world. The oral cavity witnesses the development of variety of red and white lesions many of which are associated with tobacco use. These lesions often precede the development of frank cancerous lesions known as potentially malignant disorders.The increasing prevalence of Oral cancer and Potentially Malignant Disorders in India has led to an increased need for diagnostic and prognostic markers which are reliable, easy to perform and do not need expensive equipment. Therefore,we felt that there is urgent need for the development of the new technologies in the field of the cytology to identify the most accurate site for biopsy in potentially maligdisorders. **Material and methods:** A total of 30 Patients with clinically diagnosed Potentially Malignant Disorders was taken from archives of deparment.4 smears were taken from each patient and fixed. The sample was divided into following 2 major groups:GROUP 1 (Conventional cytology) : which are further categorized on basis of staining as:Group1a) Acridine Orange stain,Group 1b) Feulgen Acriflavine stainII)GROUP 2(Liquid based cytology): which were further categorized on the basis of staining as: Group 2a) Acridine Orange stain, Group 2b) Feulgen Acriflavine stain. **Results**:Among 40 samples of conventional cytology47.50%were found positive and 21 (52.50%) were found negative.out of these positive cases (60.00%) were positive for Acridine orange whereas(35.00%) were positive for Feulgen Acriflavine.Among 40 samples of liquid based cytology, total 13 (32.50%) were found positive and 27 (67.50%) were found negative. Of these 8 (40.00%) were positive for Acridine orange and 5 (25.00%) were positive for Feulgen Acriflavine staining.Therefore, in our study, almost 50 % of theslides made by conventional cytology were positive as compared to only 32% of slide made by liquid based cytology.Out of total 40 samplesboth conventional and liquid based cytology showed clear background for 9 (50.00%) samples each.Out of total 40 samples stained with PAP stain, 19 (47.50%) showed positive uniformity of distribution and 21 (52.50%) showed lack of uniformity of distribution. Among 19 (47.50%) positives, 8 (40.00%) samples were with conventional cytology and 11 (55.00%) were of liquid based cytology. **Conclusion:** Modified techniques like liquid based cytology have been reported to be useful tool for screening oral premalignant and malignant lesions. We attempted to evaluate the efficacy of this technique using fluorescent staining.In our hands liquid based cytology failed to establish any significance advantages over conventional cytology.Among the fluorescent stains, we found Acridine orange to show better results than Acriflavine Feulgen. It's lesser cost and ease of use warrants its consideration as an adjective to Exfoliative cytology.

Key words: Exfoliative Cytology, fluorescent stains, Oral cancer.

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INTRODUCTION

Oral cancer is a major public health problem in much of South East Asian [1], as well as certain region of eastern and western Europe, Latin America, Caribbean countries, Melanesia . Although high incidence zone in Asia (India, Sri Lanka, Pakistan, Bangladesh, China – Taiwan) contribute over one third (37.5%) of the total global burden. The oral cavity witnesses the development of variety of red and white lesions many of which are associated with tobacco use. These lesions often precede the development of frank cancerous lesions known as potentially malignant disorders[2]. Precancerous lesions with dysplasia have shown a 12.3% rate of malignant transformation over a period of 0.5 – 16 years[3]. One of the most common potentially malignant disorders is the leukoplakia. However, it is very pertinent to remember that Leukoplakia is a clinical term, and that these lesions show varying histology and biologic behaviour. The increasing prevalence of Oral cancer and Potentially Malignant Disorders in India has led to an increased need for diagnostic and prognostic markers which are reliable, easy to perform and do not need expensive equipment. Although detection at the early stage significantly reduces the cancer, specific morbidity & mortality[7], oral cancer are mainly detected at the later stage which affect 5 year survival despite improvements in treatment aspect. Despite of the recent advances in the treatment modalities, the survival rate of the patients with oral cancer has not significantly improved. Therefore,we felt that there is urgent need for the development of the new technologies in the field of the cytology to identify the most accurate site for biopsy in potentially malignant disorder especially moderate to severe dysplasia's which are most susceptible to progress into invasive squamous cell carcinoma. In the oral cavity, exfoliative cytology is of great value, however it is not as a accepted as a confirmatory diagnostic aid by the Oral Pathologists community, as it was very subjective, and inter- and intra-observer variations were significant screening tool for premalignant and malignant changes in the epithelium. In an attempt to overcome these disadvantages, as the procedure fulfilled the need of being rapid, easy and economical, numerous variations of exfoliative cytology came up such as the use of fluorescent stains and liquid based cytology.

Liquid- based cytology (LBC)has significant advantages over conventional exfoliative cytology resulting in slides with high cellularity dispersed in a homogeneous thin layer[11]. Fluorescence microscopy has been studied over normal microscopy for screening of cancer cells as these cancer cells is based upon the high protein synthesis of cancerous tissue. In fluorescence microscopy Acridine orange and AcriflavineFeulgen is used as histochemical basic fluorochrome dye with specific affinity for nucleic acids[13]. Therefore in our study we evaluate the efficacy Liquid based cytology in the diagnosis of Oral Potentially Malignant Disorders and also compared the efficiency of Acridine Orange and Feulgen Acriflavine fluorescent stains in the diagnosis of Oral Potentially Malignant Disorders.

Material and methods:

A total of 30 Patients with clinically diagnosed Potentially Malignant Disorders was taken from the department of Oral Medicine and Oral Surgery of Swami Devi Dyal dental college and hospital, Barwala. Informed consent was taken from the patient.4 smears was taken from each patient and fixed. The sample was divided into following 2 major groups:

I) **GROUP 1** (Conventional cytology) : This group comprises of 2 smears taken with help of conventional cytology which are further categorized on basis of staining as:
Group1a) Acridine Orange stain
Group 1b) Feulgen Acriflavine stain

II) **GROUP 2**(Liquid based cytology): This group comprises of 2 smears taken with help of Liquid Based Cytology which were further categorized on the basis of staining as:
Group 2a) Acridine Orange stain
Group 2b) Feulgen Acriflavine stain

Histopathological examination was carried out in the same subjects for confirmation of dysplasia.

Inclusion criteria:Males in the age group of 25 – 40 yrs, Patients having white lesions in the buccal mucosa which was confirmed by Histological diagnosis.

Exclusion criteria:White lesions diagnosed as specific entities other than premalignancy.Individuals with contributory medical history like cardiovascular diseases, hypertension and diabetes mellitus which can alter lipid profiles.

The results of the slides were tabulated for Acridine orange and Feulgen Acriflavine. Liquid Based Cytology was then compared with those from Conventional Cytology with regards to the following criteria:

- Even distribution of the smear

Uniformity of staining

- Absence of overlapping of cells

RESULTS:

GROUPS			Subgroup		Total
			Acridine orange	Feulgan acriflavin	
Conventional cytology	+ve	count	12	7	19
		% within subgroup	60.0%	35.05%	47.5%
	Total	Count	20	20	40
		% within subgroup	100.0%	100.0%	100.0%
Liquid based cytology	+ve	Count	8	5	13
		% within subgroup	40.0%	25.0%	32.5%
	-ve	Count	12	15	27
		% within subgroup	60.0%	75.0%	67.5%
	Total	Count	20	20	40
		% within subgroup	100.0%	100.0%	100.%
Conventional cytology= 0.113, liquid based cytology = 0.311 (p value)					

Table 1 Among 40 samples of conventional cytology, total 19 (47.50%) were found positive and 21 (52.50%) were found negative. Of these 12 (60.00%) were positive for Acridine orange and 7 (35.00%) were positive for Feulgen Acriflavine staining. 8 (40.00%) samples were found negative for Acridine orange and 13 (65.00%) were negative for Feulgen Acriflavine staining. Among 40 samples of liquid based cytology, total 13 (32.50%) were found positive and 27 (67.50%) were found negative. Of these 8 (40.00%) were positive for Acridine orange and 5 (25.00%) were positive for Feulgen Acriflavine staining. 12 (60.00%) samples were found negative for acridine orange and 15 (75.00%) were negative for feulgen acriflavine staining.Therefore, in our study, almost 50 % of theslides made by conventional cytology were positive as compared to only 32% of slide made by liquid based cytology.

GROUPS			Group		Total
			Conventional cytology	Liquid based cytology	
Clear background	+ve	count	9	9	18
		% within subgroup	45.0%	45.0%	45.0%
	-ve	Count	11	11	22
		% within subgroup	55.0%	55.0%	57.5%
	Total	Count	20	20	40
		% within subgroup	100.0%	100.0%	100.%
(p value) =1.000					

Table 2 Out of total 40 samples stained with PAP stain, 18 (45.00%) showed clear background and 22 (55.00%) did not show clear background. Both conventional and liquid based cytology showed clear background for 9 (50.00%) samples each.

GROUPS			Group		Total
			Conventional cytology	Liquid based cytology	
Uniformity of distribution	+ve	count	8	11	19
		% within subgroup	40.0%	55.0%	47.0%
	-ve	Count	12	9	21
		% within subgroup	60.0%	45.0%	52.5%
	Total	Count	20	20	40
		% within subgroup	100.0%	100.0%	100.%
(p value)=.342					

Table 3 Out of total 40 samples stained with PAP stain, 19 (47.50%) showed positive uniformity of distribution and 21 (52.50%) showed lack of uniformity of distribution. Among 19 (47.50%) positives, 8 (40.00%) samples were with conventional cytology and 11 (55.00%) were of liquid based cytology.

DISCUSSION:

It has for long been recognized that histopathologic examination of the biopsy tissue is the Gold standard for diagnosis. However, due to the increasing incidence of oral cancer, there is a growing need for techniques which are simple,quick, economic, and can be performed without the need for much equipment. However, a lack of consistency of the slides made repeatedly, along with the basic subjective nature of the technique hampered its widespread acceptance.

Keeping the increasing need for a rapid as well as accurate diagnostic procedure in a developing country like ours, which may be performed with minimal equipment, we proposed to undertake a study combining the use of fluorescent stains with liquid based cytology in an attempt to combine the benefits of both. For our study, we used Acridine Orange and Fuelgen Acriflavine staining in slides prepared by liquid based cytology as compared to those prepared by the conventional technique. However, we found the technique for liquid based cytology to be more cumbersome and technique sensitive.Also, there was a need to prepare special test tubes for the same which had a layer of wax on to which the cells would collect centrifugation. The process was also technique sensitive,as we did not get good result after centrifugation of fifteen minutes at the rate of 800rpm,but the results were better with centrifugation at the rate 3000rpm for 5minutes.Added to this was the need for using methyl alcohol as a preservative, which is not readily available in all laboratories in our hand slides prepare with the use of methyl alcohol showed lysis of cell,so we switched to ethyl alcohol which then gave in better result.

In our study of histologically proven oral epithelial dysplasia we found more positive result with conventional cytology than with liquid based cytology. The parameters established for assesing the quality of slide like nuclear stain, clear background and lack of overlapping of cells more similar in both techniques showed almost 50 percent positive and 50 percent negative findings.

We also found more positive staining in Acridine Orange in conventional as well as liquid based cytology as compared to Feulgen Acriflavine staining. In our study, Liquid based cytology failed to give as good results as compared to conventional cytology. In liquid based cytology, sample is first placed in fixative and then processed for further processing instead of making slides directly as in conventional cytology procedure which is time consuming and cumbersome.

CONCLUSION

The increasing prevalence of cancer has fulfilled the need for a screening tool which may reliably be used in remote areas which lack modern facilities. Modified techniques like liquid based cytology have been reported to be useful tool for screening oral premalignant and malignant lesions. We attempted to evaluate the efficacy of this technique using fluorescent staining. We faced some challenges in standardizing in techniques, and came to the conclusion that the technique followed elsewhere cannot be replicated word by word and needs to be standardized locally. Moreover, in our hands liquid based cytology failed to establish any significance advantages over conventional cytology. Rather contrary, to an exception, we found conventional cytology to score over liquid based cytology in some aspects. Moreover, it also was easy to perform with rapid PAP test and re establish itself as a useful screening tool.Among the fluorescent stains, we found Acridine orange to show better results than Acriflavine Feulgen. It's lesser cost and ease of use warrants its consideration as an adjective to Exfoliative cytology. To summarize we would like to put forward that Exfoliative cytology, a useful tool, still needs to go long way before it can be accepted as a universal tool for detection of cancer and pre-cancerous lesions. However, the use of Fluorescent stains in conjunction with exfoliative cytology is definitely a step in the right direction.

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