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RESEARCH ARTICLE

RAPID DIAGNOSTIC METHOD BY SEDIMENT CYTOLOGY FOR IDENTIFICATION OF ORAL NEOPLASMS BY RESIDUAL CELLS

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ABSTRACT

Aim: To determine the diagnostic value of sediment cytology in early diagnosis of oral neoplasms. **Material and Methods:** Twenty oral biopsy specimens diagnosed of Oral squamous cell carcinomas were received in 10% formalin fixative will be analyzed for the cytological study. The material required for the study is left over formalin from specimen bottles in which the biopsies are received. Cytological smears are prepared by centrifuging the fixatives in which the biopsy specimens are received and stained with modified Papanicolau stain (PAP). All specimens were analyzed for malignancy and intensity of expression were compared. Statistical analysis was done using Chi Square test to compare the intensity of expression in different study parameters based on the cellularity in malignant cases. **Results:** All the 20 cases were adequately cellular and other cytological parameters were altered with increase in cellularity showing malignancy. On comparison of intensity of expression of nuclear pleomorphism was 6.190 with P-Value of 0.04, cellular pleomorphism was 6.190 with P-Value of 0.04 and hyperchromatic nuclei was 19.333 with a P-value of 0.001 which was statistically significant. **Conclusion:** Our study showed all cases were malignant with increase in cellularity all cytological parameters were altered. On comparing the intensity of expression of nuclear pleomorphism, cellular pleomorphism and hyperchromatic nuclei showed statistical differences. In laboratory for preliminary diagnosis of oral biopsies sediment cytology can be an alternate methodology in histopathology

INTRODUCTION

Oral squamous cell cancer is a global health problem with increasing mortality rates (1). Detecting oral malignant and potentially malignant lesions in early stages dramatically affects survival rates. Unfortunately, 50% of patients have regional or distant metastases at the time of diagnosis, which reflects a significant diagnostic delay.(2) Rapid and early diagnosis is essential to gain the benefits of initiating the treatment early. Cytological examination provides useful information for guiding therapeutic strategy.(3)The scope of cytology in the early diagnosis of neoplastic lesions has markedly increased. Sediment Cytology involves the study of smears prepared from sediment of the biopsy specimen fixatives so that a provisional diagnosis can be made as early as possible. Analysis of the sediment of the fixative and interpretation of the cytological picture with relevant clinical and radiological data allows diagnosis in a couple of hours (4).

Being rapid, simple, cheaper and easy, sediment cytology may provide early diagnostic value in such cases with the help of preparation of smears of sediments from the biopsy specimen fixatives, which have exfoliated cells from the biopsy cut surfaces. Sediment cytology is more suitably coined as biopsy sediment cytology because it is acquired from fixatives in which the biopsy specimens are received.

Examination of the sediment of fixatives and analysis of the cytological image with relevant clinical and radiological data allow judgment in a couple of hours. Sediment cytology has been effectively assessed in variety of lesions such as cervix and breast, esophagus and stomach, bladder, lung, bone lesions and ovarian neoplasms(1,5). To the best of our knowledge, there are very few number of studies conducted on the sediment cytology in OSCC. Hence, the purpose present study was conducted to estimate the efficiency of sediment cytology in biopsy of OSCC.

MATERIALS AND METHODS

This is a prospective observational study was conducted in the Department of Oral Pathology and Microbiology, Rajarajewari Dental College and Hospital, Bangalore from February 2019 to January 2020. Total 20 oral lesions were reported and included in present study Inclusion criteria: 10% formalin fixed biopsies, punch biopsy, incisional biopsy and excisional biopsies, measuring more than 1×1 cm in size, received in cases with OSCC. Exclusion criteria: Tissue received after more than 24 hours of biopsies were excluded from the study.

Twenty oral biopsy specimens received in 10% formalin fixative which were clinically diagnosed as malignant lesion were analyzed for this cytological study. The material required for this study was left over formalin from specimen bottles in which the biopsies were received. The received specimen was then transferred to a container with fresh fixative. The fluid from the original container was centrifuged at 3000 rpm for 5 min. The supernatant was discarded, and the sediment was washed twice in normal saline. The sediment was used to prepare smears on albuminized slides. Albumin coated slides were used as it ensured better adherence of cells. Smears were fixed in 95% ethyl alcohol and then stained by Papanicolaou (PAP) stain and mounted. Cytological smears were evaluated and reported as presence or absence for malignancy depending on the cytological criteria. further all the smears were evaluated for cellularity, nuclearcytoplasmic ratio, nuclear and cellular pleomorphism and hyperchromatic nuclei. Statistical analysis was done using Chi Square test to compare the intensity of expression in different study parameters based on the cellularity in malignant cases. The level of significance (P-Value) was set at P<0.05

RESULTS

The smears were assessed for presence or absence of malignancy wherein all 20 cases showed malignancy. All the cases were adequately cellular and other cytological parameters like Nucleus: Cytoplasm ratio (Figure 1), nuclear pleomorphism (Figure 2), cellular pleomorphism (Figure 3) and hyperchromatic nuclei (Figure 4) were altered with increase in cellularity. On comparison of intensity of expression of Nuclear: Cytoplasm ratio statistically significant results were not obtained (Figure 5) Comparison of intensity of expression of nuclear pleomorphism (Figure 6), cellular pleomorphism (Figure 7) and hyperchromatic nuclei (Figure 8) showed statistically significant results (Table 1)

DISCUSSION

Oral SCC is an extremely frequent human cancer with cancer being the most common among men and the third most common among women in India.(6). Delayed diagnosis is the main cause of the high morbidity/mortality of oral cancer.(7)The crucial factors for patient survival rates depends on early detection, timely and appropriate treatment. There are various techniques such as exfoliative cytology, liquid based cytology, Vital staining, Oral CDx brushstest, Chemiluminescent, tumour organoids. (8-10). In spite of various techniques applied so far, cytology is proved to be distinctly and timely method for detection of oral cancer. SC is the novel technique wherein smear is prepared by sediment of exfoliated cells in the formalin from the cut surface of biopsy specimen.(1) It is considered to be a simple, cost effective, time saving and valuable method of examination.(2).

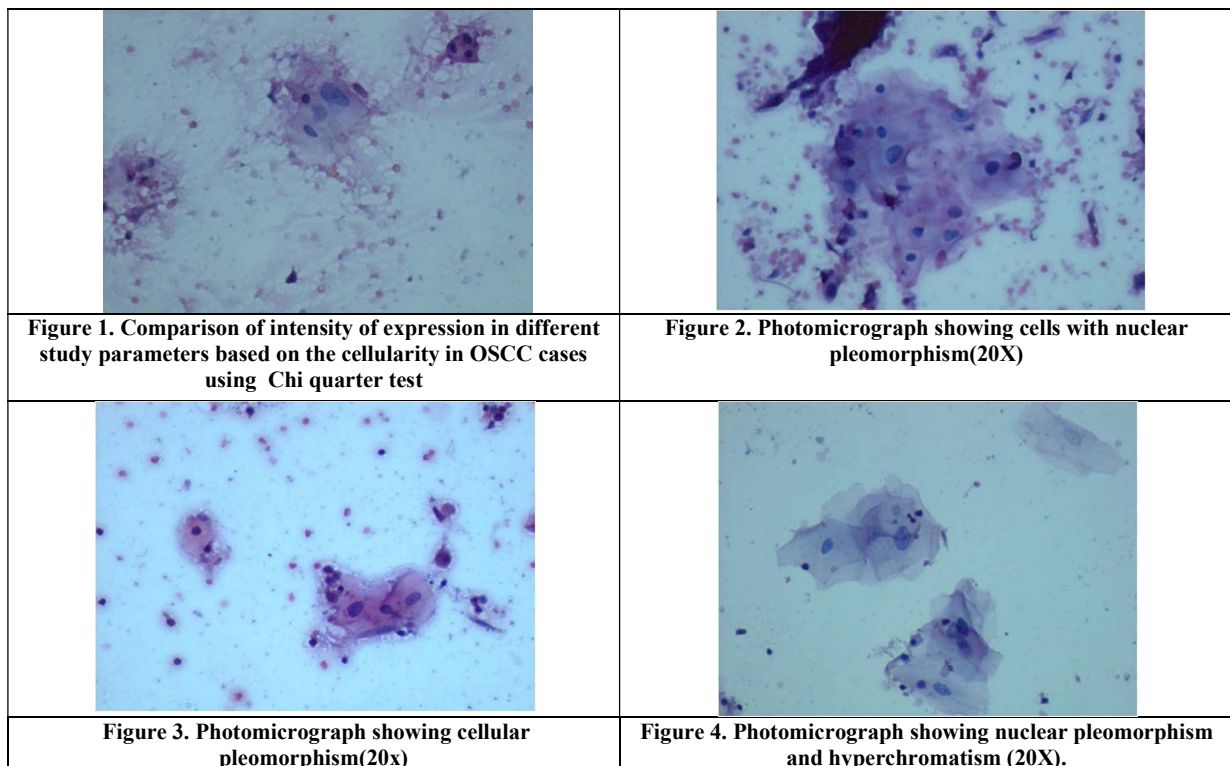


Table 1. Comparison of intensity of expression in different study parameters based on the cellularity in OSCC cases using Chi quarter test

Variables	Expression	0 - 25 Cells		26 - 50 Cells		51 - 75 Cells		> 75 Cells		χ ² Value	P-Value
		n	%	n	%	n	%	n	%		
N:C ratio	Mild	0	0%	3	100%	5	71.4%	5	50%	2.732	0.26
	Moderate	0	0%	0	0%	2	28.6%	5	50%		
	Severe	0	0%	0	0%	0	0.0%	0	0%		
Nuclear pleomorphism	Mild	0	0%	3	100%	3	42.9%	2	20%	6.190	0.04*
	Moderate	0	0%	0	0%	4	57.1%	8	80%		
	Severe	0	0%	0	0%	0	0.0%	0	0%		
Cellular pleomorphism	Mild	0	0%	3	100%	3	42.9%	2	20%	6.190	0.04*
	Moderate	0	0%	0	0%	4	57.1%	8	80%		
	Severe	0	0%	0	0%	0	0.0%	0	0%		
Hyperchromatic nuclei	Mild	0	0%	3	100%	0	0.0%	1	10%	19.333	0.001*
	Moderate	0	0%	0	0%	7	100.0%	5	50%		
	Severe	0	0%	0	0%	0	0.0%	4	40%		

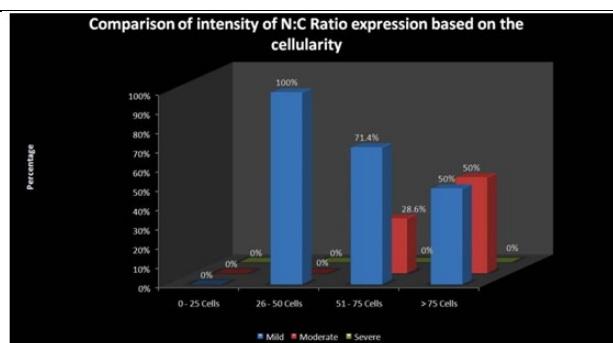


Figure 5. Graph Showing comparison of intensity of Nuclear cytoplasmic ratio expression based on cellularity

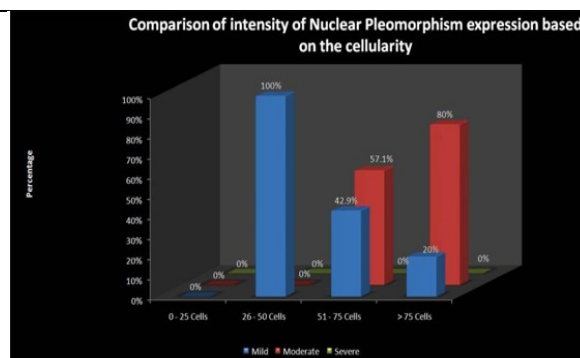


Figure 6. Graph showing comparison of intensity of nuclear pleomorphism expression based on cellularity

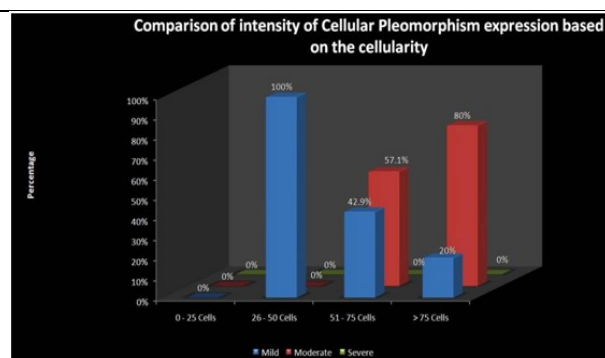


Figure 7. Graph showing comparison of intensity of Cellular Pleomorphism expression based on cellularity

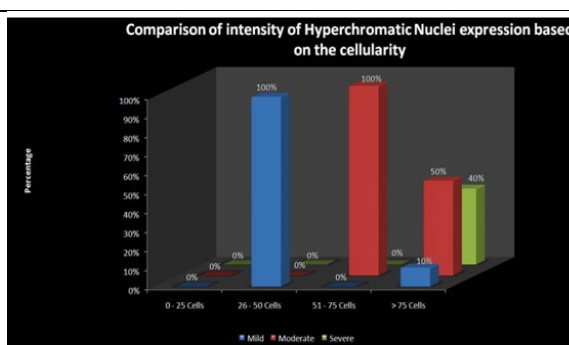


Figure 8. Graph Showing comparison of intensity of hyperchromatic nuclei expression based on cellularity

SC was considered an effective tool in early diagnosing malignant bone neoplasm as well with a accuracy rate of 79%. (4)A study suggested that SC is more sensitive and specific because nuclear details are well maintained because of better fixation and neoplastic cell is well examined as there is nopyring of inflammatory cells.(11)SC has been effectively assessing in variety of lesions such as breast, cervix, esophagus, stomach, bladder, lung, bone lesions, and ovarian neoplasms. (1) In our study a new technique in rapid diagnosis of oral neoplasm's is adopted.

Apart from rapidity of diagnosis, the other advantage of this technique is that it is a simple process that does not require any additional armamentarium. In this technique not only, small biopsy can be utilized but also provisional diagnosis can be made by fragments in the formalin of biopsy specimen. In our study we have stained cytosmear with PAP stain. PAP stain is worldwide used stain focally used for oral and cervical cancer screening wherein it yields polychromatic stained cells with evident nuclear and cytoplasmic feature. Furthermore, the PAP staining technique used is routinely available, long lasting and cost effective (12). Cytopathological assessment is considered one of the precise, objective and reproducible technique which leads to early diagnosis of OSCC. During cytopathological analysis all twenty cases showed alteration in cellular and nuclear architecture which were suggestive of 100% malignancy. Similar results were obtained in a study carried to evaluate role of sediment cytology in oral neoplasms which showed diagnostic accuracy of 85% (1). Further studies were carried out to evaluate the role of sediment cytology in bone lesions got an accuracy of 79% (4) and a study achieved a diagnostic accuracy of 90.3% in ovarian neoplasms though these studies were not based on oral neoplasms the results were in accordance to our study (3). In our study there was altered nuclear cytoplasmic ratio, nuclear pleomorphism, hyperchromatic nuclei and cellular pleomorphism which is suggestive of malignant changes. This is in accordance with a study which showed similar results though the method was different and they concluded that these findings have a practical utility in early detection

and diagnosis of the OSCC patients (13-14). The limitation of the study was that the cytological smears only categorized as malignant and features suggestive of malignancy but a conclusive diagnosis was not possible and a future study with a large sample size is imperative.

CONCLUSION

With the limitations of the study our present study showed all cases were malignant and with increase in cellularity all cytological parameters were altered. Statistically significant results were seen on comparing the intensity of expression of nuclear pleomorphism, cellular pleomorphism and hyperchromatic nuclei. The results revealed that technique gives 100% diagnostic accuracy in case of oral malignant lesions. Biopsy sediment cytology is a good compliment and alternative to regular histopathology in the study of oral biopsies. The procedure is simple, inexpensive and rapid and can be utilized with potential benefit in any laboratory for a preliminary diagnosis. More studies should be conducted with larger sample size to appraise these techniques as screening method for malignancy.

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