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Original Article

Cytomorphometric Analysis of Buccal Squames in Oral Squamous Cell Carcinoma, Different Stages of Oral Submucous Fibrosis and its Comparison to Healthy Individuals

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ABSTRACT
Objective: Oral cancer is one of the major causes of death all over the world. Diagnosing it in the earlier stages is important. Here a cytomorphometric evaluation is used to obtain information on cellular and nuclear events. The objective of the present study is to determine the variation in cellular area, nuclear area, cellular diameter, nuclear diameter and their ratios (NCA/NCD) respectively in healthy individuals, Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma patients. **Methods:** Exfoliated buccal mucosal cells collected from study subjects (n = 90) from three groups and stained using rapid Papanicolaou stain. Photomicrograph of 50 non overlapping cells captured at 40× magnification with a digital opt scopes microscope image analysis software. Image analysis was performed to obtain cellular diameter (CD), cellular area (CA), nuclear diameter (ND), nuclear area (NA), nuclear to cellular area (NCA) and nuclear to cellular diameter (NCD). These values were statistically compared among the groups using one-way analysis of variance (ANOVA) and Unpaired t test. **Results:** Study showed a significant reduction in the cellular diameter and cellular area. Increase in the nuclear diameter and nuclear area and their ratios in second and third group. Conclusion: Cytomorphometric changes could be the earliest indicators of cellular and nuclear alterations and could serve as a useful adjunct in the early diagnosis of premalignant lesions and conditions.
Key words: Oral Squamous Cell Carcinoma, Oral Submucous Fibrosis, Exfoliative Cytology, Rapid Papanicolaou.

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INTRODUCTION

In India, oral cancer is a major health problem which accounts for 30-40% of all cancers diagnosed and is the sixth common cause of death in males and seventh most common condition in females[1]. Majority of oral cancers arise from long standing potentially malignant lesions[2].Oral precancerous lesion and conditions such as oral leukoplakia and oral submucous fibrosis (OSMF) have a high rate of transformation to oral cancer[3]. Earlier detection of premalignant and malignant lesions can be done by exfoliative cytology which is a simple, non-invasive procedure. In exfoliative cytology, the quantitative parameters are objective and reproducible, they may be important aids in the making of a cytopathologic diagnosis. One such quantitative parameter is morphometry. The smear can be analyzed both quantitatively and qualitatively by the technique of exfoliative cytology. With advancements in the field of quantitative oral exfoliative cytology, various parameters such as nuclear size, cellular size, nuclear-to-cellular diameter, nuclear to cellular area are some of the parameters which are significant in the evaluation of oral lesions[4]. The variations obtained in these parameters have been attributed to exposure to carcinogenic agents like tobacco and Paan chewing. The concept of cellular or nuclear alteration on exposure to varying forms of tobacco and paan can be best explained by reviewing the nature of cellular response to stimuli from the end products of different types of usage[5]. There is little

mention in the literature of the quantitative cytologic assessment of the effects of gutkha and paan chewing on normal oral mucosa. No reports have been mentioned about the cytomorphometric assessment in different stages of oral submucous fibrosis and in these three sites of oral squamous cell carcinoma (lesional OSCC, 5mm way from the lesion and contralateral buccal mucosa cases in OSCC). Exfoliative technique has been applied to examine the cellular and nuclear changes induced by chronic gutkha and paan chewing in buccal mucosa as revealed by quantitative cytomorphometry. Thus, the aim of the present study was to analyze cytomorphometric parameters such as ND, CD, NA, CA, NCD, NCA smears in healthy individuals, different stages of oral submucous fibrosis and in patients of oral squamous cell carcinoma from three different sites (lesion, 5mm away from the lesion and contralateral buccal mucosa).

MATERIALS AND METHODS

Patients with different stages of oral submucous fibrosis (n=30) according to the mouth opening staging by Kiran et al, from different sites of oral squamous cell carcinoma (from lesion, 5 mm away from the lesion and contralateral buccal mucosa) (n=30) and healthy individuals (n=30) constituted the study group. Patient with alcohol habit, history of systemic illness and those on medication were excluded from the study. Following informed consent, scrapings were obtained using cytobrush, moistened with normal saline. Cells were spread onto a glass slide and fixed with 95% alcohol for 15 minutes. All cytological smears were stained by Papanicolaou staining technique using commercially available staining kit Rapid PAP. The morphometric analysis of stained smears was done under research light microscope (Olympus). Images of the stained cells were captured under objective of X40 magnification with digital camera (MAG CAM DC5 MP camera). 50 cells with well-defined borders were randomly selected and morphometric analysis for 6 parameters namely NA, CA, CD, NA, NCD, NCA (Table 1) were made using opt scopes microscope image analysis software (Fig.1). Unpaired "t'' test was used for comparing the parameters between multiple groups and one-way ANOVA was used to compare the parameters within the group.

RESULTS

The mean values of NA, CA, ND, CD are shown in Table 2. The mean value of ND, NA was highest in lesional oral squamous cell carcinoma followed by stage III OSMF, 5 mm away from lesion in OSCC, stage II OSMF, stage I OSMF, contralateral buccal mucosa in cases of OSCC and least in healthy individuals. Conversely, the CD & CA decreased maximum in lesional OSCC followed by stage III OSMF, 5 mm away from lesion in OSCC, stage II OSMF, contralateral buccal mucosa in cases of OSCC, stage I OSMF and minimal changes were seen in healthy individuals.

The mean of the NCA & NCD ratios (Table 3) signifies that the NCD and NCA ratios were highest in lesion group of OSCC followed by stage III OSMF, 5 mm away from lesion in OSCC, stage II OSMF, stage I OSMF, contralateral buccal mucosa in cases of OSCC and least in healthy individuals.

The comparison of mean values of parameters NA, CA, ND, CD, NCA & NCD shows statistically significant difference among the healthy individuals and the OSCC group (lesional OSCC, 5mm away from the lesion and contralateral buccal mucosa) as shown in Table 4 using unpaired "t'' test. The mean values of above-mentioned parameters showed statistically significant difference among the healthy group and stage II & III of oral submucous fibrosis. however, there was no statistically significant difference between Stage I OSMF and healthy group as shown in Table 5.

Table 6 shows a comparison of the mean of NA, CA, ND, CD, NCA & NCD values between different stages of oral sub mucous fibrosis and lesional OSCC, statistically Significant difference was observed in all values.

The results were statistically significant amongst different stages of Oral submucous fibrosis when compared with contralateral buccal mucosa and 5 mm away in cases of oral squamous cell carcinoma (Table 7 & 8).

The one-way Anova –F table was used for all the variables such as NA, ND, CA, CD, NCA & NCD to compare the intra- group (within group) for different sub groups of OSCC and OSMF (Table 9).

Table 1: CYTOMORPHOMETRIC ANALYSIS

S. No	Parameters	
1.	Nuclear Diameter (ND)	The ND & CD were measured using a digitalized cursor with interactive measurement tool by tracing two perpendicular lines (maximum and minimum diameter), which were measured by the software and then mean values were calculated.
2.	Cellular Diameter (CD)	
3.	Nuclear Area (NA)	The nuclear and cellular outlines were traced using a digitalized cursor with an interactive measurement tool and the software calculated the area.
4.	Cellular Area (CA)	
5.	Nuclear to cellular area (NCA)	NCA = NA/CA
6.	Nuclear to cellular diameter (NCD)	NCD=ND /CD

TABLE-2: MEAN & STANDARD DEVIATION OF CELLULAR DIAMETER, NUCLEAR DIAMETER, CELLULAR AREA & NUCLEAR AREA FOR HEALTHY GROUP, OSCC & OSMF GROUP

S.NO.	TYPE OF GROUPS		MEAN± S.D.			
			CELLULAR DIAMETER	CELLULAR AREA	NUCLEAR DIAMETER	NUCLEAR AREA
1	HEALTHY GROUP (30)		67.16±1.57	2956.04±95.80	9.10±.466	62.65±3.28
2	OSCC (30)	LESIONAL (30)	53.86±3.51	2312.03±96.86	10.99±.753	84.56±2.65
		5 MM AWAY from lesion in OSCC(30)	57.92±2.75	2414.49±95.80	9.86±.502	69.46±2.62
		CONTRALATERAL B.M. (30)	65.23±2.533	2902.04±95.80	9.20±.696	63.12±2.65
3	OSMF (30)	STAGE 1 OSMF (10)	65.20±1.59	2906.08±101.25	9.45±.248	63.23±2.414
		STAGE 2 OSMF (10)	60.38±1.95	2616.12±101.25	9.724±.386	69.494±1.988
		STAGE 3 OSMF (10)	53.91±.903	2312.05±101.25	10.10±.749	71.76±2.414

TABLE-3: MEAN & STANDARD DEVIATION OF RATIO'S OF CELLULAR DIAMETER & NUCLEAR DIAMETER AND CELLULAR AREA & NUCLEAR AREA FOR HEALTHY GROUP, OSCC & OSMF GROUP

S.NO.	TYPE OF GROUPS		MEAN± S.D.	
			RATIO (ND/CD)	RATIO (NA/CA)
1	HEALTHY GROUP (30)		0.14±.007	0.02±.001
2	OSCC (30)	LESIONAL (30)	0.205±.018	0.04±.00
		5 mm AWAY From the lesion in OSCC (30)	0.171±.013	0.03±.00
		Contralateral buccal mucosa in cases of OSCC(30)	0.141±.013	0.02±.00
3	OSMF (30)	STAGE 1 OSMF (10)	0.145±.003	0.022±.001
		STAGE 2 OSMF (10)	0.161±.006	0.027±.002
		STAGE 3 OSMF (10)	0.187±.013	0.031±.001

TABLE-4: COMPARISON BETWEEN HEALTHY GROUP & OSCC FROM THREE DIFFERENT SITES FOR CELLULAR AREA, NUCLEAR AREA, CELLULAR DIAMETER, NUCLEAR DIAMETER, RATIOS OF NA/CA & ND/CD (BY Unpaired t TEST)

S.NO.	PAIR OF SUB - GROUPS	PROBABLE VALUE OF INDEPENDENT "t" TEST					
		CELLULAR AREA	NUCLEAR AREA	CELLULAR DIAMETER	NUCLEAR DIAMETER	RATIO (NA/CA)	RATIO (ND/CD)
1	HEALTHY GROUP (n=30) & LESIONAL OSCC (n=10)	.0002* P<.01(SIG.)	.0001* P<.01(SIG.)	.0010* P<.01(SIG.)	.0031* P<.01(SIG.)	.0021* P<.01(SIG.)	.0001* P<.01(SIG.)
2	HEALTHY GROUP (n=30) & 5 MM AWAY from lesion in OSCC (n=10)	.0000* P<.01(SIG.)	.0001* P<.01(SIG.)	.0000* P<.01(SIG.)	.0000* P<.01(SIG.)	.0010* P<.01(SIG.)	.0000* P<.01(SIG.)
3	HEALTHY GROUP (n=30) & CONTRALATERAL B.M. in cases of OSCC (n=10)	.0000* P<.01(SIG.)	.0011* P<.01(SIG.)	.0000* P<.01(SIG.)	.0000* P<.01(SIG.)	.0012* P<.01(SIG.)	.0002* P<.01(SIG.)

* shows a significant difference at .01 level of significance,
** shows no significant difference at .01 level of significance

TABLE-5: COMPARISON BETWEEN HEALTHY GROUP & DIFFERENT STAGES OF OSMF FOR CELLULAR AREA, NUCLEAR AREA, CELLULAR DIAMETER, NUCLEAR DIAMETER & RATIOS OF NA/CA, ND/CD (BY Unpaired t TEST)

S. NO.	PAIR OF SUB - GROUPS	PROBABLE VALUE OF INDEPENDENT "t" TEST					
		CELLULAR AREA	NUCLEAR AREA	CELLULAR DIAMETER	NUCLEAR DIAMETER	RATIO (NA/CA)	RATIO (ND/CD)
1	HEALTHY GROUP (n=30) & STAGE 1 OSMF (n=10)	.0987** P>.01(N.S.)	.1154** P>.01 (N.S.)	.1158** P>.01 (N.S.)	.1038** P>.01 (N.S.)	.1044** P>.01 (N.S.)	.0939** P>.01 (N.S.)
2	HEALTHY GROUP (n=30) & STAGE 2 OSMF (n=30)	.0002* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0000* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0011* P<.01 (SIG.)	.0000* P<.01 (SIG.)
3	HEALTHY GROUP (n=30) & STAGE 3 OSMF (n=10)	.0001* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0000* P<.01 (SIG.)	.0000* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0001* P<.01 (SIG.)

* shows a significant difference at .01 level of significance,
** shows no significant difference at .01 level of significance

TABLE 6: COMPARISON BETWEEN OSCC FROM LESIONAL AREA & DIFFERENT STAGES OF OSMF FOR CELLULAR AREA, NUCLEAR AREA, CELLULAR DIAMETER, NUCLEAR DIAMETER & RATIOS OF NA/CA, ND/CD (BY Unpaired t TEST)

S. NO.	PAIR OF SUB - GROUPS	PROBABLE VALUE OF INDEPENDENT "t" TEST					
		CELLULAR AREA	NUCLEAR AREA	CELLULAR DIAMETER	NUCLEAR DIAMETER	RATIO (NA/CA)	RATIO (ND/CD)
1	LESIONAL OSCC (n=10) & STAGE 1 OSMF (n=10)	.0011* P<.01 (SIG.)	.0021* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0013* P<.01 (SIG.)	.0031* P<.01 (SIG.)	.0011* P<.01 (SIG.)
2	LESIONAL OSCC (n=10) & STAGE 2 OSMF (n=10)	.0010* P<.01 (SIG.)	.0011* P<.01 (SIG.)	.0014* P<.01 (SIG.)	.0022* P<.01 (SIG.)	.0012* P<.01 (SIG.)	.0042* P<.01 (SIG.)
3	LESIONAL OSCC (n=10) & STAGE 3 OSMF (n=10)	.0002* P<.01 (SIG.)	.0010* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0011* P<.01 (SIG.)	.0004* P<.01 (SIG.)	.0034* P<.01 (SIG.)

* shows a significant difference at .01 level of significance,
** shows no significant difference at .01 level of significance

TABLE 7: COMPARISON BETWEEN 5 MM AWAY FROM LESION IN OSCC & DIFFERENT STAGES OF OSMF FOR CELLULAR AREA, NUCLEAR AREA, CELLULAR DIAMETER, NUCLEAR DIAMETER & RATIOS OF NA/CA, ND/CD (BY Unpaired t TEST)

S. NO.	PAIR OF SUB -GROUPS	PROBABLE VALUE OF INDEPENDENT "t" TEST					
		CELLULAR AREA	NUCLEAR AREA	CELLULAR DIAMETER	NUCLEAR DIAMETER	RATIO (NA/CA)	RATIO (ND/CD)
1	5 MM AWAY from lesion in OSCC (n=10) & STAGE 1 OSMF (n=10)	.0000* P<.01 (SIG.)	.0011* P<.01 (SIG.)	.0012* P<.01 (SIG.)	.0021* P<.01 (SIG.)	.0031* P<.01 (SIG.)	.0011* P<.01 (SIG.)
2	5 MM AWAY from lesion in OSCC (n=10) & STAGE 2 OSMF (n=10)	.0011* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0004* P<.01 (SIG.)	.0022* P<.01 (SIG.)	.0021* P<.01 (SIG.)
3	5 MM AWAY from lesion in OSCC (n=10) & STAGE 3 OSMF (n=10)	.0001* P<.01 (SIG.)	.0002* P<.01 (SIG.)	.0011* P<.01 (SIG.)	.0003* P<.01 (SIG.)	.0041* P<.01 (SIG.)	.0003* P<.01 (SIG.)

* shows a significant difference at .01 level of significance,
** shows no significant difference at .01 level of significance

TABLE 8: COMPARISON BETWEEN CONTRALATERAL B.M. IN CASES OF OSCC& DIFFERENT STAGES OF OSMF FOR CELLULAR AREA, NUCLEAR AREA, CELLULAR DIAMETER, NUCLEAR DIAMETER & RATIOS OF NA/CA, ND/CD (BY Unpaired tTEST)

S. NO.	PAIR OF SUB - GROUPS	PROBABLE VALUE OF INDEPENDENT "t" TEST					
		CELLULAR AREA	NUCLEAR AREA	CELLULAR DIAMETER	NUCLEAR DIAMETER	RATIO (NA/CA)	RATIO (ND/CD)
1	**CONTRALATERAL B.M. in cases of OSCC (n=10) & STAGE 1 OSMF (n=10)**	.0000* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0000* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0031* P<.01 (SIG.)	.0021* P<.01 (SIG.)
2	**CONTRALATERAL B.M. in cases of OSCC (n=10) & STAGE 2 OSMF (n=10)**	.0022* P<.01 (SIG.)	.0012* P<.01 (SIG.)	.0001* P<.01 (SIG.)	.0014* P<.01 (SIG.)	.0022* P<.01 (SIG.)	.0001* P<.01 (SIG.)
3	**CONTRALATERAL B.M. in cases of OSCC (n=10) & STAGE 3 (OSMF)**	.0021* P<.01 (SIG.)	.0032* P<.01 (SIG.)	.0014* P<.01 (SIG.)	.0013* P<.01 (SIG.)	.0041* P<.01 (SIG.)	.0013* P<.01 (SIG.)

* shows a significant difference at .01 level of significance,
** shows no significant difference at .01 level of significance

TABLE 9: THE ONE-WAY ANOVA –F TABLE FOR COMPARING THE INTRA- GROUP (WITHIN GROUP) FOR DIFFERENT SUB GROUPS OF OSCC, OSMF FOR CELLULAR DIAMETER/AREA, NUCLEAR DIAMETER/AREA & THEIR RATIOS

S.NO.	SOURCE OF VARIATION	D.F.	F-VALUE	P-VALUE	F CRIT	SIGNIFICANCE
1	Between Groups FOR OSCC (FOR C. D.)	2	112.72	0.0000	4.8578	**P<.01 (SIG. DIFF.)**
2	Between Groups FOR OSCC (FOR N. D.)	2	56.51	0.0000	4.8578	**P<.01 (SIG. DIFF.)**
3	Between Groups FOR OSCC (FOR RATIO IN DIAMETERS)	2	113.69	0.0000	4.8578	**P<.01 (SIG. DIFF.)**
4	Between Groups FOR OSMF (FOR C. D.)	2	136.47	0.0000	4.8578	**P<.01 (SIG. DIFF.)**
5	Between Groups FOR OSMF (FOR N. D.)	2	4.13	0.0271	4.8578	**P<.01 (SIG. DIFF.)**
6	Between Groups FOR OSMF (FOR RATIO IN DIAMETERS)	2	64.6	0.0002	4.8578	**P<.01 (SIG. DIFF.)**
7	Between Groups FOR OSCC (FOR C. A.)	2	322.46	0.0003	4.8578	**P<.01 (SIG. DIFF.)**
8	Between Groups FOR OSCC (FOR N.A.)	2	522.34	0.0001	4.8578	**P<.01 (SIG. DIFF.)**
9	Between Groups FOR OSCC (FOR RATIO IN AREA'S)	2	651.08	0.0002	4.8578	**P<.01 (SIG. DIFF.)**
10	Between Groups FOR OSMF (FOR C. A.)	2	86.06	0.0000	4.8578	**P<.01 (SIG. DIFF.)**
11	Between Groups FOR OSMF (FOR N. A.)	2	37.56	0.0002	4.8578	**P<.01 (SIG. DIFF.)**
12	Between Groups FOR OSMF (FOR RATIO IN AREA)	2	134.64	0.0001	4.8578	**P<.01 (SIG. DIFF.)**

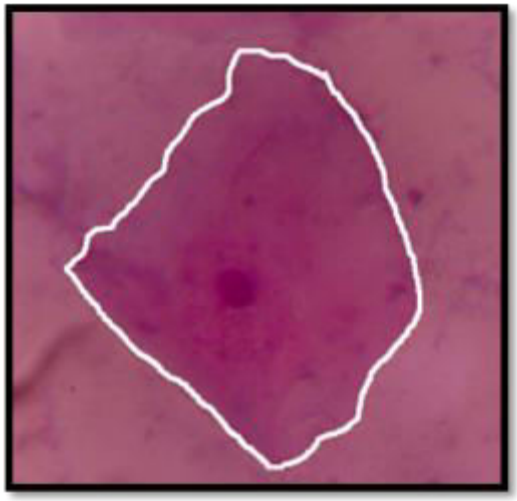

Fig.1: Measurement of PAP stained cell using Opt scopes microscope image analysis software.

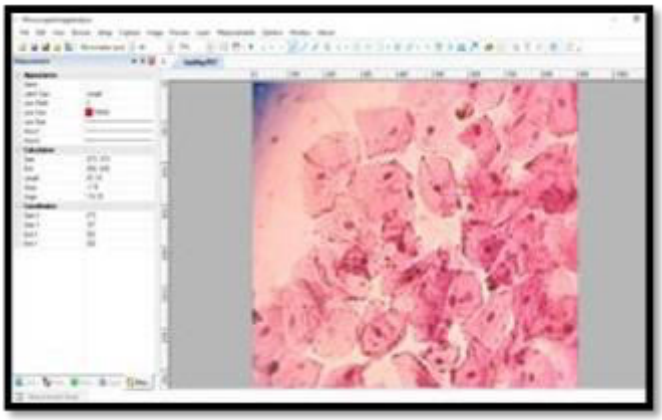

Fig. 2: Photomicrograph showing tracing of cellular diameter of a cell from exfoliative cytology in healthy individuals using Opt Scopes image analysis software (Rapid PAP stain, 40x).

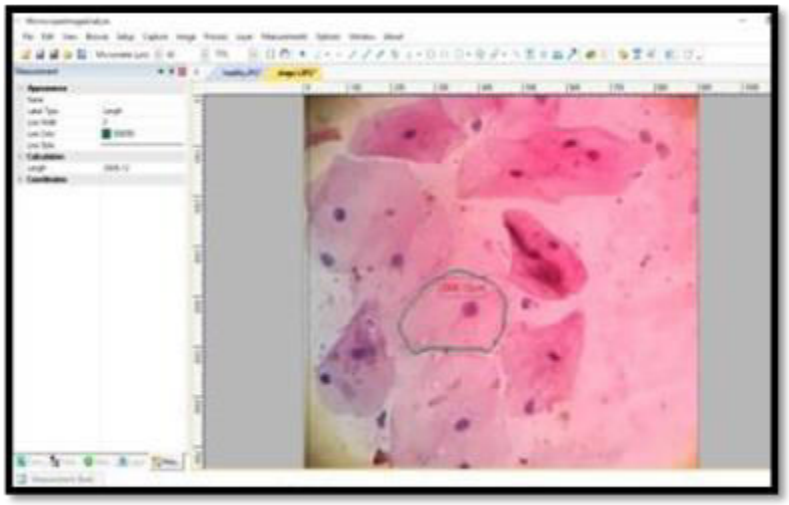

Fig. 3: Photomicrograph showing tracing of cellular area of a cell from exfoliative cytology in Stage I OSMF using Opt Scopes image analysis software (Rapid PAP stain, 40x).

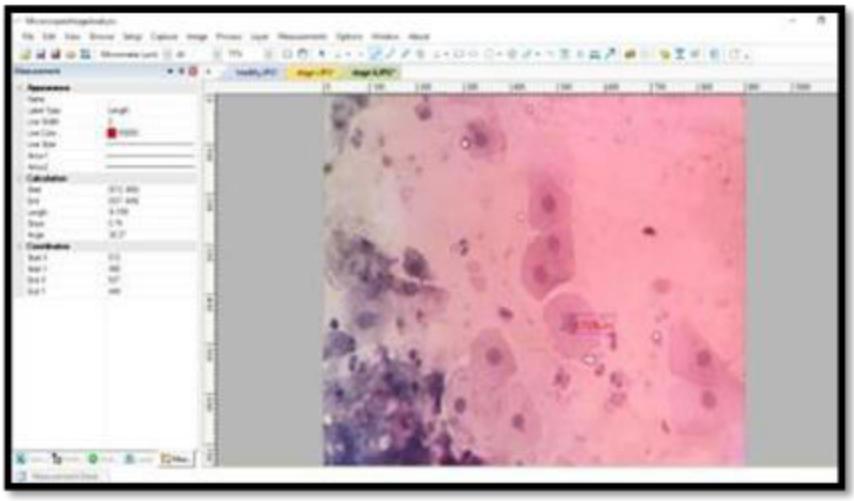

Fig. 4: Photomicrograph showing tracing of nuclear diameter of a cell from exfoliative cytology in Stage II OSMF using Opt Scopes image analysis software (Rapid PAP stain, 40x).

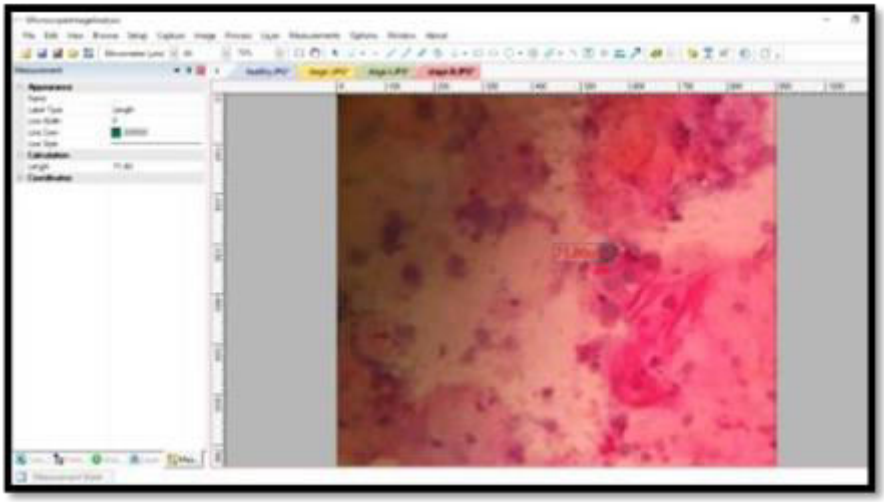

Fig 5: Photomicrograph showing tracing of nuclear area of a cell from exfoliative cytology in Stage III OSMF using Opt Scopes image analysis software (Rapid PAP stain, 40x).

DISCUSSION

Quantitative cytomorphometric assessment of exfoliated buccal mucosal cells has shown measurable changes in the cells obtained from malignant and premalignant lesions. Morphometric changes may refer to mechanical injury, chronic inflammation, constant exposure to carcinogen, nutritional deficiency etc[4]. Cowpe JG et al showed that exfoliative cytology can be used to detect the malignant changes through evaluation of nuclear to cellular size in Papanicolaou stained smears with the technique of Planimetry[6]. Since then a number of studies have been carried out using the quantitative cytomorphometric technique to evaluate the influence of external factors on cellular size & nuclear size and their ratios, but the results are varied and controversial. Literature search showed no reports on cytomorphometric analysis of buccal squames in different stages of OSMF and in cases of OSCC from 5 mm away from the lesion and in contralateral buccal mucosa.

The present study revealed that the mean value of ND was highest in lesional Oral squamous cell carcinoma (10.99±.753,) followed by stage III OSMF (10.10±.749), 5 mm away from lesion in OSCC (9.86±.502), Stage II OSMF (9.724±.386), Stage I OSMF (9.45±.248), Contralateral buccal mucosa in cases of OSCC (9.20±.696) and least in Healthy group (9.10±.466). Mean value of NA was highest in lesional OSCC (84.56±2.65) followed by Stage III OSMF (71.76±2.414), Stage II OSMF (69.494±1.988), 5 mm away from lesion in OSCC (69.46±2.62), Stage I OSMF (63.23±2.414), Contralateral buccal mucosa in cases of OSCC (63.12±2.65) and least in Healthy (62.65±3.28). Similar findings of an increase in ND & NA have been reported in a study conducted by

Sharma et al. This increase in nuclear dimension is related to an increase in the nuclear contents required for replication.

Goregon[7], Acharya[4], Bhavasar[8] also found significant difference in the nuclear parameters.

Results of our study revealed that the mean values of CD & CA decreased from healthy individuals (CD= 67.16±1.57, CA= 2956.04±95.80) to OSMF & OSCC. Mean value of CA decreased maximum in lesional OSCC (2312.03±96.86) followed by Stage III OSMF (2312.05±101.25), 5 mm away from lesion in OSCC (2414.49±95.80), Stage II OSMF (2616.12±101.25), Contralateral buccal mucosa in cases of OSCC (2902.04±95.80), Stage I OSMF (2906.08±101.25). Mean value of CD decreased from lesional OSCC (53.86±.903) followed by Stage III OSMF (53.91±3.51), 5 mm away from lesion in OSCC (57.92±2.75), Stage II OSMF (60.38±1.95), Stage I OSMF (65.20±1.59), Contralateral buccal mucosa cases in cases of OSCC (65.23±2.5333).It was in accordance with the study of Acharya et al where significant decrease in CD, CA was noted from healthy controls to gutka chewers to OSCC cases. The amount of cytoplasm produced in relation to nucleoplasm is less resulting in decreased cellular dimensions.

Khandelwas S[9], Weigum[10] and Hande[11] also found significant difference in the mean cellular dimensions.

The present study signifies that the NCD ratio was highest in lesion group of OSCC (.205±.018 in ND/CD) followed by stage III OSMF (.187±.013), 5 mm away from lesion in OSCC (.171±.013), Stage II OSMF (.161±.006), Stage I OSMF (.145±.003), Contralateral buccal mucosa in cases of OSCC (.141±.013), Healthy (.14±.007). The NCA ratio is highest in lesional group of OSCC (.04±.00) followed by stage III OSMF (0.31±.001), 5mm away from the lesion in OSCC (.03±.00), Stage II OSMF (.027±.002), Stage I OSMF (.022±.001), Contralateral buccal mucosa cases in OSCC (.02±.00) and healthy group (.02±.001).Similar results were found in a study by Nivia et al where the ratio was found to have increased from normal mucosa to oral SCC with the highest value.

Van Oijen (1998) reported that decrease in the cell diameter and increase in the nuclear diameter are two significant morphologic changes that occur in actively proliferating cells. Firstly, there is reduced ability for the cell to differentiate into its more mature cell type. Additionally, the amount of cytoplasm that cell makes decrease in relative to nucleoplasm, so that the cell diameter decreases and there is an actual increase in the nuclear size. This may be related to an increase in the nuclear contents required for replication. As a result, the nuclear to cellular ratio increases at times to an extreme degree[12].

Our findings were in contrast to the study conducted by Diniz Freitas M, Garcia-Garcia A, Crespo-Aabelleira A, Martins-Carneiro JL, Gandara-Rey JM (2004) which revealed that neither cellular area, nuclear area, nuclear to cellular area ratio differed when OSCC patients where compared with healthy group. [13]

Contrary to our results Cowpe, Longmore and Green (1988) concluded in their study that nuclear size is not always a clear cytologic indication of dysplastic change. They found nuclear size to vary with advancing age in normal oral squames and did not find an increase in size in abnormal lesions.[14]

Statistically significant difference was seen between lesional OSCC, smears from 5mm away from the lesion and contralateral buccal mucosa cases in OSCC when compared in different parameters. Among the three clinical stages of oral submucous fibrosis there was statistically significant difference in the mean values of various parameters except that there was no statistically significant difference in the mean values of various parameters between Stage I OSMF and healthy individuals.

On the basis of our findings and above-mentioned studies we support the view that quantitative assessment of buccal exfoliative smears could be the prognostic indicators of malignancy.The results in the present study suggests that cytomorphometric analysis of oral mucosal cells can be used as an early indicator of malignant alterations.

CONCLUSION

Cytomorphometric changes in cellular and nuclear morphology could aid in early diagnosis of malignancy. Increase in nuclear and reduction in cellular dimensions acts as an early morphometric alteration in oral submucous fibrosis and oral squamous cell carcinoma patients. Based on the following data it can be said that CA, NA, CD, ND, NCD & NCA ratios could possibly be sensitive parameters in the diagnosis of oral premalignant and malignant lesions.

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