



## Original Research Article

## Effect of smoking on cytomorphology of buccal mucosal cells: Can it be a non invasive tool to detect precancerous changes in smokers?

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## ABSTRACT

**Introduction:** Oral cancer is one of the cancers highly prevalent in India affecting people with habit of using tobacco and alcohol. By detecting cytomorphological changes in buccal mucosa could increase the chances of earlier detection of premalignant and malignant lesions and thereby early intervention.

**Aim:** To assess and compare the cytomorphological changes in buccal mucosa cells amongst smoker and non-smoker group and assess these findings in smokers with duration of exposure to smoking by dividing them as per pack year groups.

**Materials and Methods:** This comparative study was carried out on 80 individuals consisting of 40 male cases having history of Cigarette or Bidi smoking and 40 controls as per inclusion and exclusion criteria. For finding effect of smoking exposure severity, smokers were further divided in three groups based on Pack Year like group 1 with pack Year <5, group 2 with pack year 5-10 and group 3 with pack year >10. After taking written informed consent, sample was taken from buccal mucosa and then slide was stained with papnicolaou stain. Total of 100 cells were scanned to find cytomorphological changes like Binucleated cell, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic vacuoles and micronuclei presence. All data were noted and subjected to statistical analysis.

**Results:** It was found that there was significant difference for Mean values of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis and micronuclei in buccal mucosa of smokers and non-smokers. We found that pyknosis, cytoplasmic granules and micronuclei were presented with significance in smoker groups having pack year <5, 5-10 and >10.

**Conclusion:** The present study indicates that almost all cytomorphological findings were high in smokers than non-smokers. All above findings we got were present in healthy mucosa of smoker and such findings are also observed in increased severity in premalignant conditions like leukoplakia. So it is possible to pick up these findings earlier by non-invasive method like exfoliative cytology and it can be used as an adjunct tool for mass screening due to its non-invasive nature, easier, cheaper and reproducible way for examination of oral cytology.

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## 1. Introduction

Among various diseases, cancer has become a big threat to human beings globally with half of all cancers occurring in developing and under-developed countries.<sup>1</sup> Amongst all, oral cancer affects lip, mouth and tongue is one of the ten most common cancers in the world and it mostly affects males.<sup>2</sup> Oral cancer is a major problem in the Indian

subcontinent ranking amongst top three types of cancer in the country.<sup>3</sup> Epidemiological studies show that the risk of developing oral cancer is five to nine times greater for smokers than for non-smokers.<sup>4,5</sup> The variation in incidence and pattern of oral cancer is due to regional prevalence of risk factor like tobacco use and alcohol.<sup>6</sup>

Oral cancer is one of the cancers highly prevalent in India and mostly affects lower middle class people indulged in habit of using tobacco and alcohol. Despite accessibility of oral cavity for visual examination, and even though oral

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cancers and premalignant lesions have well-defined clinical diagnostic features, oral cancers in India are presented in advanced stage in 60-80% cases leading to reduce survival of patients.<sup>7,8</sup> Factors like detection of cases at advanced stage, high use of tobacco and alcohol amongst people, poor health care facility, unawareness of people towards self-examination of mouth cavity, highly costly treatment make oral cancer control unsuccessful. The precancerous lesion (Leukoplakia, erythroplakia) can be detected 15 years prior to their change to an invasive carcinoma. Intervention at this stage may result in regression of lesion.<sup>3</sup> So it is necessary to develop a technique which can detect all these changes earlier and our study is aimed to detect such oral changes by applying exfoliative cytology method. Here we will use this method to find out cytomorphological changes in cells of buccal mucosa of smoker and compare that with non-smoker.

Oral exfoliative cytology involves microscopic analysis of cells collected from the surface of the oral mucosa. It is a simple diagnostic technique which could increase the chances of earlier detection of premalignant and malignant lesions and thereby early intervention.<sup>9</sup> There are various studies show that cytomorphological findings obtained by an exfoliative cytology can be used as an early predictor of premalignant lesion of oral mucosa.<sup>9-11</sup> This technique has advantages like easy and fast implementation, painless, adequate diagnostic value, non-invasiveness, low cost and reproducibility. The purpose of this study was to evaluate and compare cytomorphological changes of the exfoliated buccal mucosal cells in smokers with results obtained for non-smokers and to correlate these changes with duration of smoking by using pack year formula.

## 2. Materials and Methods

This comparative study was carried out on 80 individuals aged 25-70 years selected randomly from indoor patients of Medicine Department of Tertiary care hospital and rest procedure was performed in department of Anatomy. Before starting the study, prior permission had been taken from the institutional ethics committee. Patients were grouped as according to Pack year formula<sup>12,13</sup> to find total exposure to tobacco due to cigarette and Bidi.

Pack Year= Number of cigarettes per day x Number of year smoked/20

Pack Year= Number of bidis per day x Number of year smoked/20 x 0.25

The subjects were divided into 3 groups as follows:

Group 1: Patients with Pack Year <5

Group 2: Patients with Pack Year 5-10

Group 3: Patients with Pack Year >10

Women were not included in the study due to cellular changes during menstruation, after menopause and also due to the possibility of pregnancy and other hormonal changes.<sup>14</sup> Cases (smokers) were non anaemic and non

diabetic male patients with clinically healthy mucosa and having only history of smoking and not received radiotherapy or chemotherapy in last 1 month. Controls (Non-smoker) were consisted of 40 subjects with no history of smoking and without any systemic illness /anaemia and Diabetes.

Patients wearing denture, under or followed radiation or chemotherapy, alcoholic, anaemic, diabetic, having malignant, premalignant lesion of oral cavity, addicted to other form of tobacco or alcohol or having painful oral lesions were excluded from study. After explaining procedure and taking informed consent, personal data about name, age, history of smoking, any systemic diseases of patients were collected. The sample area of buccal mucosa was dried using a piece of sterile gauze and exfoliated buccal cells were obtained from both the normal sides of cheek by scraping 3 to 4 times with a firm pressure applied by a new wooden spatula. Samples were spread on a centre of dried pre coded clean glass and then immediately fixed with fixation spray to avoid exposure to dry air. Then the slides were stained with Rapid Papnicolaou (PAP) staining<sup>15</sup> technique and examined under a microscope for various cytomorphological changes like Binucleated Cell, Pyknosis, Perinuclear Halo, Cytoplasmic granulation, Karyolysis, Karyorrhexis, Cytoplasmic vacuoles and Cells with Micronuclei were observed. (Figure 1, Figure 2) The data of smoker group was further analysed based on their subgroup according to pack year. For Cytomorphological features, 100 cells were scanned under 10 X and 40 X objective lenses and findings were noted. Obtained data were subjected to appropriate statistical.

## 3. Results

In present study, following results were obtained between smoker and non smoker. Study shows that there were 40 cases and 40 controls and Mean age of the cases was 46.7 years and that of control was 48.6 years.

Table 1 shows that mean of binucleated cells in Non-Smoker was  $0.86 \pm 0.85$  while in smoker mean of binucleated cells was  $1.47 \pm 1.33$ . Mean of pyknotic cells in Non-Smoker and Smoker was  $0.90 \pm 1.39$  and  $2.71 \pm 1.74$  respectively. Mean cells with Perinuclear Halo in Non-Smoker and Smoker was  $0.41 \pm 0.90$  and  $1.45 \pm 1.63$  respectively. Mean cells with cytoplasmic granules in Non-Smokers and smoker was  $0.49 \pm 0.92$  and  $1.60 \pm 1.50$  respectively. It shows that mean of Karyolytic cells in Non-Smoker was  $0.04 \pm 0.20$  while in Smoker mean of karyolytic cells was  $1.00 \pm 3.01$ . Mean of cells showing karyorrhexis in Non-Smoker and Smoker was  $0.02 \pm 0.14$  and  $0.41 \pm 1.00$  respectively. Mean of cells with cytoplasmic granules in Non-Smoker and Smoker was  $0.04 \pm 0.20$  and  $0.16 \pm 0.54$  respectively. Mean of cells with micronuclei in Non-Smokers and Smoker was  $0.65 \pm 0.09$  and  $3.06 \pm 2.26$  respectively.

**Table 1:** Comparison of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic granules and micronuclei amongst smoker (cases) and non-smoker(control)

Variable	Non - Smokers Mean $\pm$ SD	Smokers Mean $\pm$ SD	P Value
<b>Binucleation</b>	0.86 $\pm$ 0.85	1.47 $\pm$ 1.33	0.007 (Significant)
<b>Pyknosis</b>	0.90 $\pm$ 1.39	2.71 $\pm$ 1.74	< 0.001 (Significant)
<b>Perinuclear Halo</b>	0.41 $\pm$ 0.90	1.45 $\pm$ 1.63	< 0.001 (Significant)
<b>Cytoplasmic Granule</b>	0.49 $\pm$ 0.92	1.61 $\pm$ 1.50	< 0.001 (Significant)
<b>Karyolysis</b>	0.04 $\pm$ 0.20	1.00 $\pm$ 3.01	0.025 (Significant)
<b>Karyorrhexis</b>	0.02 $\pm$ 0.14	0.41 $\pm$ 1.00	0.004 (Significant)
<b>Cytoplasmic vacuoles</b>	0.04 $\pm$ 0.20	0.18 $\pm$ 0.55	0.149 (Non significant)
<b>Micronuclei</b>	0.65 $\pm$ 1.09	3.06 $\pm$ 2.26	< 0.001 (Significant)

There was significant difference observed between Non-Smokers and Smoker for cells with binucleation, pyknosis, perinuclear halo, cytoplasmic granules, Karyolysis, Karyorrhexis and micronuclei in buccal mucosal cells but no significant difference was found for cytoplasmic vacuoles between Smoker and Non-Smokers.

Table 2 shows that 40 cases (smokers) were divided as per pack year and Group 1 with pack year <5 had 12 case, Group 2 with pack year 5-10 had 15 cases and group 3 with pack year >10 had 13 cases of smoker. Group 1 with pack year <5 had mean pack year  $3.21 \pm 1.23$ , group 2 with pack year 5-10 had mean pack year  $7.42 \pm 1.46$  and group 3 patients with pack year >10 had mean pack year  $14.07 \pm 1.90$ .

Table 3 shows that amongst group based on pack year, there is significant difference between all pack year group for Pyknosis, cytoplasmic granules and micronuclei but no significant difference was observed for binucleation, perinuclear halo, karyolysis, karyorrhexis and cytoplasmic vacuoles findings.(Table 3 )

#### 4. Discussion

Cancer is one of the most life threatening diseases afflicting mankind. Although oral cancer is ranked fifteenth position amongst all cancer in the world, it is the third most common cancer in India leading to about 1,30,000 death due to tobacco related oral cancer.<sup>16</sup> Smoking, tobacco chewing and alcohol are main causative factors for development of oral cancer. Horrifying fact about oral cancer are its rising incidence in past decades and its diagnosis at advanced stage even having an easily accessible site for examination and diagnosis making it one of the cancers with lowest 5 year survival rate.<sup>17</sup> Hence, the early diagnosis of oral cavity cancers is of immense value in successful treatment of patients. The only way to curse problem of rising trends of oral cancer is by early detection, histopathological investigation, creating awareness for tobacco cessation and treating tobacco related oral cancer patients especially in their premalignant state which may be the only hope in reducing burden of it.<sup>16</sup>

Oral cancer mostly occurs as a result of malignant transformation of a pre-existing lesion like leukoplakia, erythroplakia and oral sub mucous fibrosis (Osmf).<sup>18</sup> Identification of an early premalignant lesion having potential to malignant transformation can improve scenario in cancer control programme.<sup>10</sup> Premalignant changes may present with findings like large and prominent nuclei, increased nuclear to cytoplasmic ratio, hyperchromatic nuclei, abnormal shaped nuclei and cells and increased mitotic activity. In some apparently healthy smokers, changes are observed in the frequency of epithelial cell proliferation, the size of nucleus and the size of nucleus in relation to cytoplasm.<sup>19</sup> Nuclear enlargement with the increased nuclear/cytoplasmic ratio, nuclear hyperchromatism, chromatin clumping with prominent nucleation, irregularity of nuclear membranes, bi- or multinucleation, increased keratinization are known to be the most important signs of cellular atypia and indicate increased cellular activity in the squamous epithelium.<sup>20</sup> Exfoliative cytology is a technique which helps to find out early cellular alteration in buccal mucosal cells in a rapid, easy and non - invasive way. Smoking induced early cellular atypia seen in squamous cells of buccal mucosa can be seen in premalignant condition and these changes can be identified by exfoliative cytology method before appearance of visible lesion.

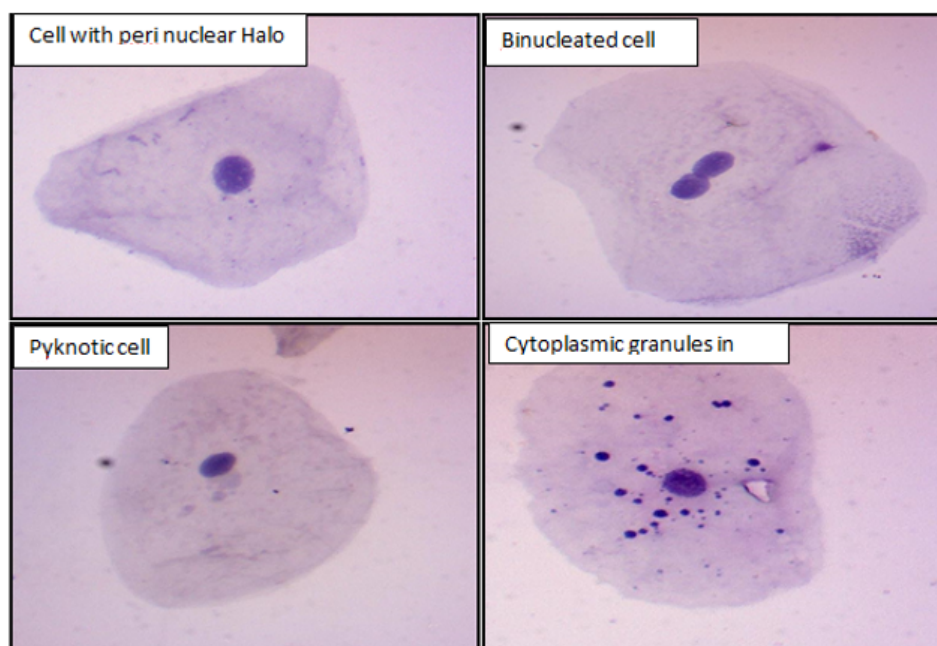
In present study, buccal mucosal cells are observed for cytomorphological changes in 40 smokers with healthy oral mucosa and compared with 40 non- smoker having healthy oral mucosa. We observed for Binucleation, Pyknosis, Cytoplasmic granules, Perinuclear halo, Karyolysis, Karyorrhexis, Cytoplasmic vacuoles and Micronuclei amongst smoker and non-smoker. Out of these except cytoplasmic vacuoles, all other parameters were significantly higher in smoker group. When we compared these findings in groups of smoker based on pack year, significant mean values of pyknosis, cytoplasmic granules and micronuclei were presented with significant difference within all three groups with maximum number of cells with these parameters were present in group 3 (pack Year >10). Findings of our study were compared with findings of other studies.

**Table 2:** Distribution of smokers (Cases) as per Pack year

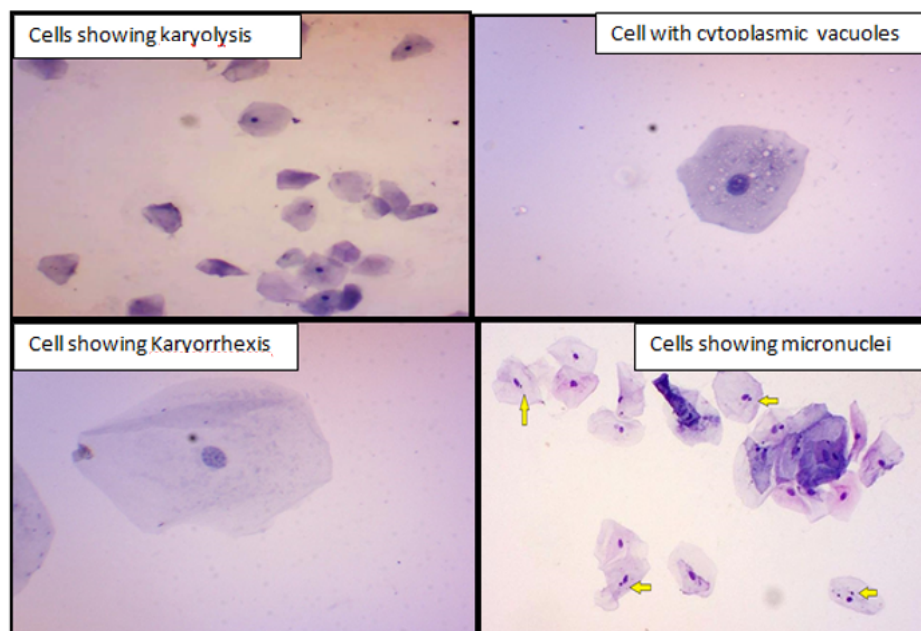
Group	Pack Year	Number of subjects (n=40)	Pack Year Mean ± SD
1.	Patients with Pack Year < 5	12 (30 %)	3.21 ± 1.23
2	Patients with Pack year < 5-10	15 (37.5%)	7.42 ± 1.46
3	Patients with Pack Year >10	13 (32.5 %)	14.07 ± 1.90

**Table 3:** Comparison of binucleation, pyknosis, perinuclear halo, cytoplasmic granules, karyolysis, karyorrhexis, cytoplasmic vacuoles and micronuclei amongst smoker groups based on pack year

Variable	Pack year <5 ( Group 1) n=12 Mean± SD	Pack year < 5-10 ( Group 2) n=15 Mean± SD	Pack year >10 ( Group 3) n=13 Mean± SD	P value (Significant/ Non significant)
Binucleation	1.47± 1.19	1.32 ± 1.00	1.65 ± 1.77	0.765 ( Not significant)
Pyknosis	2.07 ±1.44	2.11±1.15	4.00 ± 1.84	<0.001 ( Significant)
Perinuclear Halo	1.27 ±1.58	1.42 ± 1.57	1.65 ± 1.80	0.807 ( Not significant)
Cytoplasmic Granule	0.60 ± 0.74	1.63 ± 1.34	2.47 ± 1.66	0.001 ( Significant)
Karyolysis	0.27 ± 1.03	0.42 ± 1.84	2.29 ± 4.58	0.092 ( Not significant)
Karyorrhexis	0.40 ± 0.91	0.11± 0.46	0.76 ± 1.39	0.144 ( Not significant)
Cytoplasmic vacuoles	0.67 ± 0.26	0.21 ± 0.54	0.24 ± 0.75	0.417 ( Not significant)
Micronuclei	1.13 ± 1.19	2.89 ± 1.24	4.94 ± 2.38	<0.001 ( Significant)



**Fig. 1:** Showing cell with perinuclear halo, binucleated cell, pyknotic cell and cell with cytoplasmic granules



**Fig. 2:** Showing cell with karyolysis, karyorrhexis, cytoplasmic vacuoles and micronuclei

**Table 4:** Comparison for cytomorphological parameters between Previous studies with present study

Authors	Avanindr K et al. <sup>10</sup> 2014	Sharma VL <sup>21</sup> 2013	Biswas SD <sup>22</sup> 2014	Hugo V <sup>23</sup> 2015	Seifi S et al <sup>24</sup> 2014	Present Study
Variables	N= 30	N=100	N=50	N=24	N=40	N=51
Binucleation	-	7%**	29.2 ± 8.5**	-	42.9%	1.47 ± 1.33 **
Pyknosis	-	22%**	38.0 ± 13.1**	110.0 ± 33.3*	-	2.71 ± 1.74 **
Perinuclear Halo	-	-	-	-	-	1.45 ± 1.63 **
Cytoplasmic Granules	-	-	-	-	-	1.61 ± 1.50 **
Karyolysis	0 case*	50%**	24.2 ± 12.4**	14.4 ± 22.0 *	-	1.00 ± 3.01 **
Karyorrhexis	6 case*	11%**	13.8 ± 7.5**	16.3 ± 13.8 *	42.1%	0.41 ± 1.00 **
Cytoplasmic Vacuoles	12 case*	27%**	-	-	30.8 %	0.18 ± 0.55

N= Number of smokers with H/o Bidi or cigarette smoking

\*\*= values statistically significant,

- = parameter not mention

\* = values statistically insignificant or statistics not applied

Binucleated cells are formed as a consequence of cytokinetic disturbance and lead to an imbalance of the cellular DNA content in last nuclear division.<sup>25</sup> Nersesyan A et al in his study of 83 heavy smokers and 20 controls considered binucleated and broken cells in smokers more specific to find DNA damage to cell by carcinogen. Study of Khlifi R et al<sup>26</sup> showed comparison of Binucleated cells in between Head and neck cancer and normal person. They found mean binucleated cells  $5.93 \pm 2.99$  amongst 1000 cells scanned in cancer patients while in control mean binucleated cells were  $3.09 \pm 1.82$ . Yadav AS et al.<sup>27</sup> in their study of 48 smokers and 52 non-smokers showed increased mean binucleated cells in smoker in comparison

to non-smokers with significance and also showed increased frequency of binucleated cells of  $4.57 \pm 0.49$ ,  $5.11 \pm 0.45$ ,  $7.12 \pm 0.62$  amongst 1000 cells scanned respectively in 3 groups with increased number of cigarette consumption in an individual.

Pyknosis is defined as cells with small shrunken nucleus having high density of nuclear material which is intense stained all over. They may represent an alternative mechanism of nuclear disintegration different than process of karyorrhectic cell death stages.<sup>28,29</sup> Lav'inia T'ercia Magalh~aes D'orea et al<sup>30</sup> has showed that when cytological findings were compared in patients with cancer and control, it showed that apoptosis (i.e. karyorrhexis,

condensed chromatin, and pyknosis) occurred significantly more frequently in cells obtained from lesion areas of cancer patients than in cells from the control group ( $P < 0.0001$ ).

Nadaf A et al.<sup>31</sup> in which group of 50 non tobacco user showed absence of perinuclear halo while two other groups of 50 individuals with habit of any form of tobacco use and other group with 50 patients with history of tobacco use and leukoplakia showed perinuclear halo as findings in them. No other study found describing this finding amongst smoker. Jaitley S et al.<sup>32</sup> also noted that 30 patients with clinically diagnosed oral submucous fibrosis showed increased frequency of inflammatory findings like intracytoplasmic bacterial colonies, inflammatory cells, perinuclear halo, free nuclei, and indented cellular outline indicative of cytolysis in buccal mucosa than control group with no oral lesion. Study done by Kamath VV et al.<sup>18</sup> mentioned about presence of cells with micronuclei with cytoplasmic granules in smokers.

Karyolytic cells are cells in which the nucleus is completely depleted of DNA and is apparent as a ghost like image. In present study, mean karyolytic cells in smokers were  $1.00 \pm 3.01$  while in controls they were  $0.04 \pm 0.20$  showing significant difference for smoker and non-smoker. It is similar to studies done by Sharma VL<sup>21</sup> et al., Biswas SD et al.<sup>22</sup> and Hu go V et al.<sup>23</sup> had reported significantly increased frequency of karyolysis amongst smoker than control. Nersesyan A et al.<sup>25</sup> studied effect of different types of filtered cigarette smoking on buccal mucosal cells of 83 heavy smoker to 20 non-smoker in terms of nuclear anomalies including micronuclei (MN), broken eggs (BE), binucleates (BN), condensed chromatin (CC), karyorrhexis (KR), karyolysis (KL) and pyknosis (P) and found significant increase in all finding. When we compared Karyolysis finding as per pack year grouping, we found that mean value of karyolytic cells were  $0.27 \pm 1.03$ ,  $0.42 \pm 1.84$  and  $2.29 \pm 4.58$  respectively in smoker with pack year  $<5$ ,  $5-10$  and  $> 10$  with highest values were present in group 3 with pack year  $> 10$ . So with increased duration of exposure, there was higher number of karyolytic cells and same findings are reported in study of Yadav AS et al.<sup>33</sup>

Cells with karyorrhexis have nuclear disintegration are seen as nuclear fragmentation leading to the eventual loss of integrity of the nucleus. In present study, mean of karyorectic cells in smoker were  $0.41 \pm 1.00$ , while in control no cells with karyorrhexis were present showing significant difference between smoker and non-smoker. These findings are similar with study done by Avandindra K et al<sup>10</sup> who reported that out of 30 smokers 6 smokers showed karyorrhexis but no control showed presence of karyorrhexis. In present study, group 1 with pack year 5 showed mean of cells with karyorrhexis were  $0.40 \pm 0.91$ , in group 2 with pack year  $5-10$  they were  $0.11 \pm 0.46$  and in group 3 with pack year  $>10$  showed  $0.76 \pm 1.39$  with no significant difference within all 3 groups. Yadav

AS et al.<sup>33</sup> also noted karyorrhexis in 3 groups based on number of cigarette smoke per day, they found results of  $1.14 \pm 0.23$ ,  $0.56 \pm 0.17$  and  $1.50 \pm 0.26$  respectively for 3 groups indicating no increased karyorrhectic cells in group smoking  $>20$  cigarette per day in compare to group with use of  $1-10$  and  $11-20$  cigarette per day. Cells with cytoplasmic vacuoles show multiple clear spherical vacuolization of variable size. They are due to partial or temporary disturbances in the cell membrane permeability.

Table 5 shows comparison mean of cells with micronuclei of present study with that of other study. As per the finding all 6 studies shows significantly higher number of cells with micronuclei in smokers in comparison to non-smoker and prove genotoxic effect of tobacco smoke. Higher numbers of mean cells with micronuclei in some studies are due to scanning of more cells than present studies.

Dr. Farah Ali Shafi<sup>36</sup> concluded that higher frequency of micronuclei directly associated with the decrease efficiency of DNA repair and increase of genomic instability. Palve DH et al.,<sup>37</sup> Sangle VA et al.,<sup>38</sup> Dindgire SL<sup>39</sup> and Lavinia Tercica Magalhaes Dorea et al.<sup>30</sup> carried out micronuclei study in oral pre malignant lesion patients and oral cancer patient and concluded increased number of nuclei in both conditions. They suggested that micronucleus assay in the buccal cell is sensitive, practical, inexpensive screening method for genetic damage in human. In a study by Yeralgudda K et al.<sup>40</sup> also reported more number of micronuclei in pre malignant lesion like leukoplakia and submucous fibrosis or squamous cell carcinoma than controls.

In present study when we compare cells with micronuclei in relation to pack year, in group 1 with pack year  $<5$  showed its value  $1.13 \pm 1.19$ , in group 2 with pack year  $5-10$  they were  $2.89 \pm 1.24$ , and in group 3 with pack year  $>10$  they were  $4.94 \pm 2.38$ . So we observed significant difference of micronuclei according to pack year groups.

When we applied post hoc test to find out whether micronuclei finding is significant amongst various groups or not, we found that it was significant between group 1 & 2, between 2 & 3 and also between 1& 3. So we can say that number of cells with micronuclei show cell damage by increasing their frequency according to severity. Similar findings of increased number of cells with micronuclei are observed in study of Yadav AS<sup>27</sup> where increasing values are seen in groups with increased frequency of cigarette about  $1-10/$  day,  $11-20/$  day and  $20/$  day respectively. Same findings were found in study of Nanderi NJ et al.<sup>34</sup> But study by Oliviear LU et al.<sup>41</sup> has reported no significant difference statistically in micronuclei or karyorrhexis among smoker, non-smoker and alcohol group but they proposed an influence of the number of cigarettes per day on micronuclei frequency.

**Table 5:** Comparison for Micronuclei parameter between previous studies with present study

Study & Year	(Number of Non-Smoker / Smoker)	Mean Value For MN (Number of cells scanned)		P value
		Control	Smoker	
Palaskar S et al. <sup>33</sup> 2010 (Pune)	15 / 15	6.13 ± 2.29 (1000 Cells)	22.07 ± 5.88	< 0.001
Nanderi NJ et al. <sup>34</sup> 2012 (Iran)	23 / 14	2.26. ± 2.17 (500 Cells)	13.9 ± 5.90	<0.002
El-Setouhy M et al. <sup>35</sup> 2013 (Egypt)	78 / 128	3.7 ± 1.6 (1000 cells)	8.0 ± 3.2	<0.001
Biswas SD et al. <sup>22</sup> 2014 (Durgapur)	50 / 50	1.4 ± 1.1 (2 - 5 field)	11.0 ± 6.6	0.021
Hugo V et al. <sup>23</sup> 2015 (Brazil)	24 / 14	0.0 ± 0.1 (2000 Cells)	0.7 ± 0.8*	< 0.05
Farha Ali Shafi <sup>36</sup> 2015 (Iraq)	44 / 46	10.18 ± 1.07 (1000 Cells)	12.89 ± 1.85	<0.05
Present study 2016	40 / 40	0.65 ± 1.09 (100 Cells)	3.06 ± 2.26	<0.001

Kamath VV et al.<sup>18</sup> compared micronuclei correlation with duration of smoking. They found group smoking for 5-10 years showed more MN count than the group smoking for <5 years and for >10 years. This may be due to variable number of cigarette or bidi smoking in individuals falling in same duration group but when it was compared with frequency of smoking it revealed that group smoking >10 cigarettes/day had high MN count than group smoking <5 or 5-10 cigarette per day. This finding is in accordance to our study.

When injury to cell happens it will either result in degenerative, Inflammatory, repair or neoplastic changes. Degenerative changes could be swelling and enlargement of cell, wrinkled nuclear margin, pyknosis or karyolysis. Inflammatory changes could be in form of nuclear enlargement, margination of chromatin, Binucleation or multinucleation, perinuclear halo or cytoplasmic vacuoles. Reparative changes can be enlarged nucleus, multinucleation, prominent nucleoli. Enlarged hyper chromatic irregular nucleus represents neoplastic change.<sup>42</sup> So tobacco smoke may activate all these mechanism and that could reason for various cytomorphological changes found in our study.

## 5. Conclusion

The present study highlights the use of oral exfoliative cytology as an effective tool in non-invasive screening of population under the risk of oral cancer as early oral cancers and precancerous lesions are often subtle and asymptomatic. Health care professional can be trained for this procedure for early detection of subtle lesion. It can be a better option in all patients contraindicated for biopsy and also as a non-invasive procedure in follow up cases of oral cancer. Even it can be useful as an educational tool in smoker for cessation of smoking and to teach hazards associated with smoking. Availability of exfoliative cytology at rural level can provide benefit to poor patients.

Future study detecting conversion of healthy oral mucosa into premalignant or carcinoma lesion in high risk group patients by using cytomorphological parameters as a screening test in large sample may be helpful. It would be prudent to conduct further research work in this area and subsequent public health implication

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None.

## 7. Conflict of interest

None

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