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Evaluation of Micronuclei in Buccal Mucosa – Comparing Smokers And Non Smokers

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***Abstract*: -** *According to WHO over 1 billion people are associated with tobacco smoking and it accounts for approximately 4 – 5 million deaths a year worldwide which is estimated to increase to 10 million by 2030. Tobacco is a known causative agent in numerous cancers one of the major being oral cancer. Buccal mucosal cells are easily accessible and their response to the carcinogenic substances in cigarette can be studied easily for risk assessment with the help of a biomarker. Biomarker is a measurable DNA and RNA characteristic that can help study biologic and pathologic damage. Micronucleus count is a potentially useful candidate for such a biomarker. Micronuclei are fragments or whole chromosomes which did not reach spindle poles during mitosis and remain encapsulated at telophase in a separate nucleus. This study showed the usefulness of micronuclei for risk assessment due to genotoxic damage in smokers in comparison to non smokers. Not only was a significant increase in micronuclei found in smokers but also an increase in micronuclei with respect to age , duration of exposure and frequency of exposure was observed. Our study concludes that post standardization micronuclei may be used as screening tool for oral cancers.*

Keywords: - *Micronuclei, Buccal smear, smokers*

I. Introduction:-

Tobacco has been considered the greatest disease producing chemical to humans. Oral squamous cell carcinoma is the most common type of cancer in oral cavity worldwide (1). Oral cancer affects 274,000 people worldwide annually (2).In the developing countries oral cancer is the 4th commonest cancer in males and 5th commonest in women. Oral cancer ranks in top 3 of all cancers in India (3). Smoking is the known to be the most significant etiological factor for oral cancer. A cigarette contains numerous cytotoxic substances such as polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, heavy metals and pesticide residues (4). The oral cavity is also one of the easily accessible areas for cytological analysis in the body. Exfoliative cytology of the oral cavity is an easy and valuable site for biomarker assay to identify chromosomal aberrations. The most important biomarker used as an indicator of structural and numerical chromosomal aberrations is Micronuclei (5). Micronucleus is a microscopically visible round or oval cytoplasmic chromatin mass in the extra nuclear vicinity which originates from aberrant mitosis and consists of eccentric chromosomes, chromatid fragments or whole chromosomes, which have failed to reach spindle poles during mitosis (6). Micronucleus has been used consistently as a biomarker for assessment of DNA damage (5, 6).

Aim: - To identify and compare presence of Micronuclei in the exfoliated epithelial cells of buccal mucosa in smokers (case) and Non smokers (control).

Objectives:-

1. To observe and calculate Micronucleus in buccal mucosal cells.
2. To compare micronucleus in smokers and non smokers.
3. To study micronuclei in relation to age, frequency and duration of smoking.

II. Materials And Method:-

A community based prospective study was carried out in the rural community around our hospital. Buccal smears were collected from a total of 100 individuals. Informed verbal consent was obtained from all the individuals. Personal particulars and history of smoking was recorded for each of them. The study group was further divided into two groups –

Group A: 50 with history of smoking
Group B: 50 with no history of smoking

Subjects were selected with the following criteria

Inclusion Criteria –

- Smoking atleast 1 pack cigarette a day for the last 5 years.
- Males older than 21 years
- Smokers without any apparent clinical oral lesion

Exclusion Criteria-

- Individuals with history of any systemic disease

After routine oral examination subjects were asked to rinse their mouth with a normal saline solution for 2 minutes to eliminate debris and excess salaiva. Cytological smears of exfoliated cells were collected from the buccal mucosa using a premoistened wooden spatula which was scraped firmly across the mucosa and smeared on precleaned glass slides. These were immediately fixed in 95% ethyl alcohol. The smears were then stained using Papanicalou method with standard procedure. The smears showing satisfactory staining with blue nuclei, pink/orange cytoplasm of the keratinized squamous cells and the blue or green staining of the cytoplasm of the non keratinized squamous epithelial cells. Micronuclei was counted in 100 epithelial cells in each slide. The micronuclei were identified by criteria given by Tolbert et al (5).

Table 1: Criteria For Identification Of Micronuclei (Tolbert Et Al)

Parameters for cell inclusion in cells to be scored	The suggested criteria for identifying Micronuclei are
Intact cytoplasm and relatively flat cell position. Little or no overlap of cells. Little or no debris Nucleus normal and intact, nuclear perimeter smooth and distinct.	Rounded smooth perimeter Less than a third of the diameter of the nucleus but big enough to discern the shape Staining intensity similar to nucleus Texture similar to nucleus Same focal plane as nucleus Absence of overlap with or bridge to the nucleus.

The number of micronuclei was counted, recorded and data was analysed.

III. Results:-

Age distribution of the smoker group and non smoker group was comparable with most common age group being 41 to 50 years. All the selected subjects were male. The age distribution of the study group is shown below in Table 2.

Table 2: Age Distribution Of The Study Groups

AGE (years)	SMOKERS		NON SMOKERS	
	Number	Percentage	Number	Percentage
21 – 30	6	12	2	4
31 – 40	10	20	10	20
41 – 50	16	32	18	36
51 – 60	14	28	15	30
61 - 70	4	8	5	10

In the smokers group 32% are in the age group of 41 – 50 and 36% in the control group are from this age group. 28% of smokers and 30% of the non smokers are between 51 – 60 Micronucleus count in smokers and non smokers are tabulated in Table 3.

Table 3: Micronucleus Count In Smokers And Non Smokers

MICRONUCLEUS COUNT	SMOKERS		NON SMOKERS	
	Number	Percentage	Number	Percentage
NIL	5	10	20	40
1 – 5	22	44	24	48
6 – 10	15	30	4	8
>10	8	16	2	4

The micronucleus count among smokers is 44% of cases showing 1 – 5 micronuclei per100 cells counted and 30% showing 6 – 10 micronuclei and 16% showed more than 10 micronuclei. Amongst the control group 48% showed 1 – 5 micronuclei and 40% showed none. Only 8% showed 6-10 micronuclei and 4% >10 micronuclei .The mean micronucleus count in the smokers group is 5.98+/- 6.16. Control group mean is 3.67 +/- 6.64.
The difference between the two groups is statistically significant with a P value less than 0.05.

Micronuclei count in relation to age was studied by dividing the study group comprising smokers into three categories < 25 years, 25 – 50 years and > 50 years. The total number of subjects in each categories with the mean micronuclei count in each is detailed in Table 4.

Table 4: Micronuclei Count In Relation To Age In Smokers

Age In Years	Total Members In the group	Mean Micronucleus Count
< 25years	12	2.9
– 50 Years	28	5.8
>50 Years	10	11.4

It is found that the mean micronucleus count increases with age with the least count of 2.9 in the below 25 years age group and the maximum of 11.4 micronuclei count in individuals above 50 years of age. An intermediate count of 5.8 is seen in the 25 – 50 years age bracket.

To study the effect on micronuclei count per 100 cells in relation to duration of smoking the smokers group was divided into individuals smoking since <6 years , 6 – 12 years and >12 years. The results obtained are shown in Table 5.

Table 5: Micronuclei Count In Relation To Duration Of Smoking

Duration of smoking in years	Number of members in the group	Mean micronuclei count
<6 years	19	3.1
6 – 12 years	16	6.25
>12 years	15	10.8

The damage in the mucosa increases with the years of smoking as depicted above. The mean micronucleus count is highest with more than 12 years of smoking at 10.8. In duration of less than 6 years the count is 3.1 and 6 – 12 years it is 6.25.

The Micronuclei count was also correlated with the frequency of smoking by dividing the smokers into 3 groups namely <6 cigarettes per day, 6 – 12 cigarettes / day and >12 cigarettes/day. The results are tabulated in Table 6.

Table 6: Micronuclei Count In Relation To Frequency Of Smoking

Frequency Of Smoking (Cigarettes/Day)	Number Of Members In the group	Mean Micronuclei Count
<6	12	2.91
6 – 12	14	4.4
>12	24	9.08

The group with increased frequency of smoking shows more micronuclei at 9.08 while the groups with <6 cigarettes per day and 6-12 cigarettes per day show a mean count of 2.91 and 4.4 respectively.

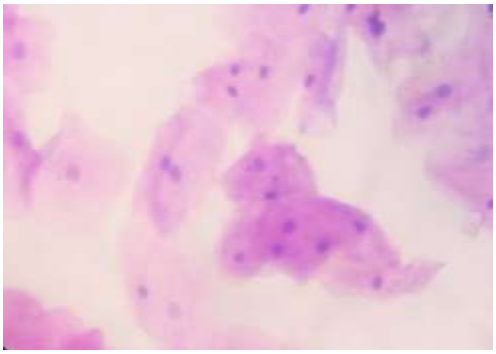

Figure1: Non smokers without MN

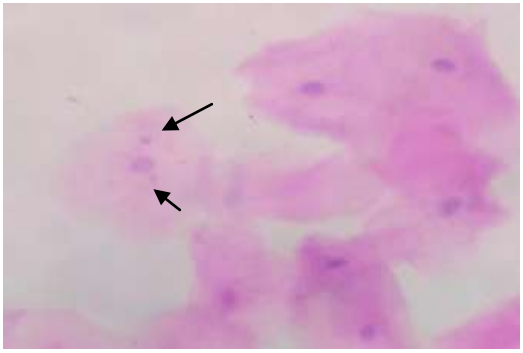

Figure 2: Smokers with two MN

IV. Discussion:-

Cancer is one of the most life threatening diseases affecting man and cigarette smoking is attributed to increase the risk for many of them including oral cancer. That is probably why the United state centre for Disease control and prevention describes tobacco use as "the single most important preventable risk to human health and an important cause of premature death world wide" (7).

The use of micronuclei assessment of exfoliative cells as a guide to early detection of genotoxic damage in human tissues is well established. Seleppa et al (8) rightly suggested that oral cavity offers a unique opportunity to define biomarkers as it permits non invasive examination in longitudinal studies of smoking. The human micronucleus project (http://www.humn.org) (9) established in 1997 has already standardizes micronucleus assay in peripheral blood lymphocytes. Casartelli et al (10) observed MN frequencies in exfoliated buccal cells – normal mucosa, precancerous lesions and squamous cell carcinoma. They observed an increase in Micronucleus count from precancerous lesions to squamous cell carcinoma suggesting its importance as a biomarker for neoplastic progression.

Sarto et al (11), Kamath et al (12), Kewan et al(13) suggest the statistically significant rise in micronucleus count in smokers with comparision to non smokers similar to that seen in our study. Kamath et al (12) found the MN count to be almost double in smokers as compared to non smokers as did we in our study. As in our results , the genotoxic injury in the form of micronucleus increases with age (12) (13).we agree with Kamath et al (12) this may be due to the compounded genotoxic effect of smoking on the mucosa for a prolonged period of time. Holland et al deduced that the genotoxic effects of substances such as tobacco and radiation are not just limited to the affected cells as it causes DNA damage which are then passed on to the daughter cells (14).The duration and frequency of smoking is also seen to induce increased injury as was seen in our study. Kewan et al attributes this to the fact the effect of increased genotoxic effect of tobacco and environmental factors on oral mucosa with increased frequency and nucleus regulates MN which is a cytological feature (13). Moreover it is observed in accordance with other studies that Papanicolaou stain is a good routine stain for micronuclei assay thereby simplifying the procedure even more (12) (15) (16). It is seen to give a clear background devoid of debris and salivary proteins.

V. Conclusion:-

Micronuclei assay is a simple procedure which can be performed using routine stains with little expertise. It is an 'internal dosimeter' which helps in determining the harm caused by carcinogenic substances (17). This method can be used to identify, monitor and educate the individuals who are found to be high risk by both habit and Micronucleus assay. By larger studies the procedure must be made objective and standardized which will enable it to be used as a powerful screening tool for oral cancers.

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