Evaluation of Micronuclei in Buccal cavity and Blood cells as a biomarker tool for Oral Cancer screening

Priyanshu Singh[1], Ankur Chhari[2], Sameena Akhter[3], Shivani Deshmukh[4], Shivani Tomar[5], Alibha Rawat[6], Shweta Azad[7], Rachna Jain[8], Sarfaraz Hanfi[9]

[1,9]*Department of Genetic Engineering, Devi Ahilya University, Indore, M.P, India2*

[2,3,4,5,6]*Department of Research & Clinical Genetics, Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah, Hills, Bhopal, 462001, Madhya Pradesh India.1*

[7,8]*Department of Pathology, Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah, Hills, Bhopal, 462001, Madhya Pradesh India.3*

***Abstract*- The aim of this study was to describe the role of Micronucleus Frequency as a biomarker tool for Oral cancer screening & DNA damage compare with healthy individuals and oral cancer patients, through Buccal cells by BCMN assay and peripheral blood culture by (*in vitro*) CBMN assay. The frequency of MN has been shown to be greatly enhanced in patients with oral precancerous lesions and cancer when compared to healthy individuals. Peripheral blood lymphocytes have also been used to evaluate systemic genotoxic effects, for which higher MN frequencies have been found among oral cancer patients. Selection of patients/ volunteers, Oral swab and peripheral blood was collected from patients/ volunteers, Blood Culture, Harvesting of Micronucleus, Washing of Cells, Air Drop Method, Preparation of Slide, Giemsa staining, Microscopic observation of micronucleus morphology which shows genotoxic effects and DNA damage . A helpful biomarker for evaluating the effects of chromosome damaging substances and a trustworthy technique for assessing chromosomal damage in peripheral blood lymphocytes by cytokinesis-block micronucleus (CBMN) assay. According to this study, BCMN and CBMN with MN had a strong positive correlation, and the frequency of MN was linked to age and cancer stage. A notable rise in micronucleus formation in cancer patients suggestive of cytotoxicity or DNA damage. According to the study, oral cancer exhibits genotoxicity that is noticeably higher than that of healthy individuals. Buccal and blood samples can be used to predict the frequency of micronuclei, enabling an early assessment of genotoxicity prior to the emergence of clinical symptoms. This could be helpful in screening of the individual who are at high risk of cancer and early detection of those who have family history of cancer as high-risk individuals, such as smokers, tobacco chewers and alcohol users, according to the study, cytogenetic damage and the development of cancer are related.**

***Index Terms*- Oral cancer screening, Buccal mucosa cells, Peripheral blood lymphocytes, Cytogenetic biomarkers, Oral squamous cell carcinoma (OSCC), Cytokinesis-block micronucleus (CBMN) assay.**

I. INTRODUCTION

One of the most common cancers in the world, oral cancer has a poor prognosis, a delayed clinical diagnosis, and costly treatment. Squamous cell carcinoma of the oral cavity (OSCC), its definition, epidemiology, oral carcinogenesis, potential malignant disorders, epithelial precursor lesions, experimental approaches, treatments, and upcoming obstacles. Early diagnosis is crucial for improved outcomes, study conducted by (Rivera C. et al., 2015). Micronuclei, or extra nuclei in biology brought on by genetic damage, are detected by light microscopy. In clinical contexts such as genotoxicity supervision, disease biomonitoring, preneoplastic disease screening, and high-risk patient identification, MN scoring demonstrates damage. (ElienBeyls, EviDuthoo et al., 2025). Human epithelial buccal cells are tested for micronucleus using the BMn assay, which assesses lifestyle factors such as exposure, smoking, alcohol use, cancer risk, neurological disorders, and accelerated aging. (Sommer et al., 2020). Because of their various changes, buccal cells—a popular assay technique for researching tobacco's genotoxic effects—show promise as biomarkers for the early detection of oral cancer. (Gopal & Padma, 2018; Sharma et al., 2018). The CBMN assay, created by Fenech and Moreley, uses Cytochalasin-B to inhibit cytokinesis and produce binucleated cells, making it a dependable technique for evaluating DNA damage in individuals. (Sioen et al., 2020). The CBMN assay is a widely used bio-monitoring tool for detecting genomic instability, in vivo genotoxic exposure, and mutagenic chemical toxicity, resulting in DNA double-strand break (Kanagaraj et al. 2023). MN is more common in the circulating lymphocytes of people exposed to genotoxic chemicals, suggesting a higher risk of cancer. (Senthil. K et al., 2015).

II. MATERIAL & METHODS

After taking approval from 'IEC' for this study samples collected from Male and female individuals of both sex, aged 18 to 60, were chosen at random for the study. Their medical history, rate of tobacco use, and interactions with other toxins were also taken into consideration during the selection process. Both internal and external witnesses witnessed the signing of the informed consent forms.

Oral swab for Buccal cell slide preparation was taken by trained nursing staff and 2ml peripheral blood was drawn from patients and normal individual in heparinized blood collection tubes Take 5 ml equal amount of RPMI 1640 media in centrifuge tubes, distribute the 2- 5 ml of blood sample in each tubes keep this centrifuge tubes in incubator for 48 to 72 hours at 37°C, shake the tubes every 24 hours (2 to 3 time). After 72 hours of incubation, 50µl Cytochalacin-B solution was added to the blood sample, incubated for 35 minutes at 37°C, and then centrifuged for 10 minutes. The tubes were then mixed with KCl, PBS, Cornoy's Fixative, and stored in the refrigerator at 4°C. After centrifuging, the tubes were cleaned and prepared with Cornoy's Fixative. Samples were put on slides that had been prepared using the Air Drop technique. Giemsa working solution was then used to stain the slides, enabling excellent nuclear membrane and chromatin staining. After that, the slides were examined under a microscope, and a Giemsa stain solution and a microscope were used to analyze the findings.

III. OBSERVATION

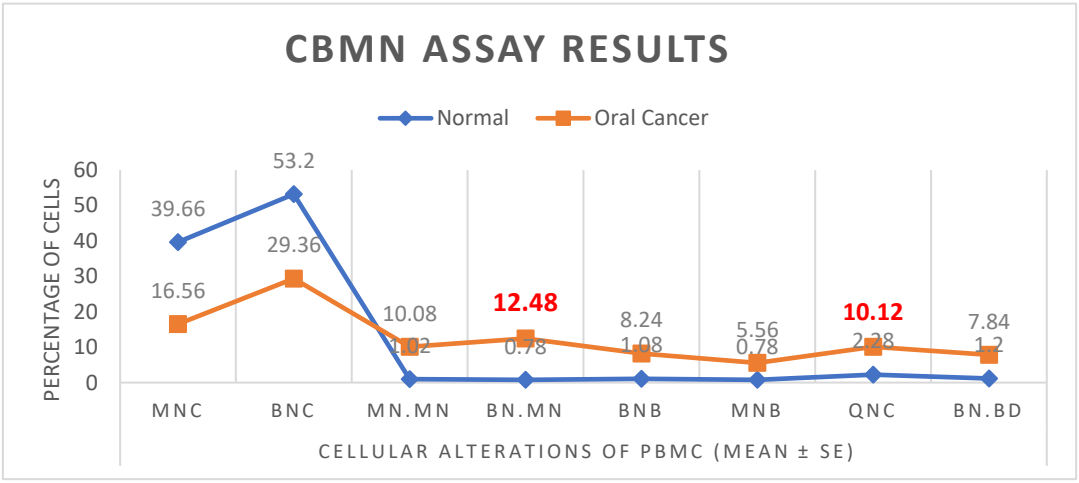
Table 01: Showing comparative CBMN assay results for both normal individual and oral cancer patients.

Groups	Cellular Alterations of PBMC (Mean ± SE)							
	MNC	**BNC**	**MN.Mn**	**BN.Mn**	**BNB**	**MNB**	**QNC**	**BN.Bd**
Normal	39.66	53.2	1.02	0.78	1.08	0.78	2.28	1.2
Oral Cancer	16.56	29.36	10.08	12.48	8.24	5.56	10.12	7.84

P Value= **0.005**, Significant level (<0.05)

Note:

MNC: Mononucleated Cell
BNC: Binucleated Cell
MN.Mn: Mononucleated Micronucleus
BN.Mn: Binucleated Micronucleus
BNB: Binucleated Bud
MNB: Mononucleated Bud
QNC: Qudra Nucleated Cell
Bn.Bd: Binucleated Bridge

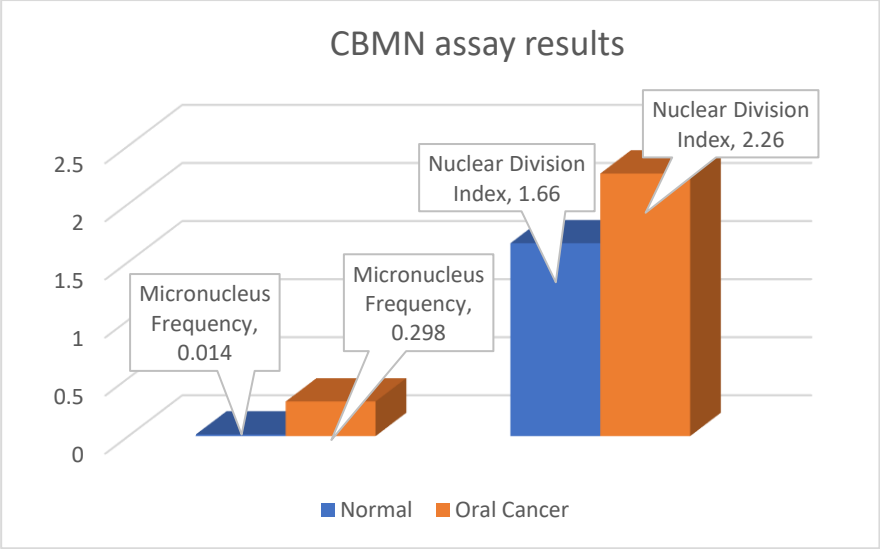


Graph: 01:

Showing cellular alteration of PBMC (Mean ±SE) of normal individual and oral cancer patients.

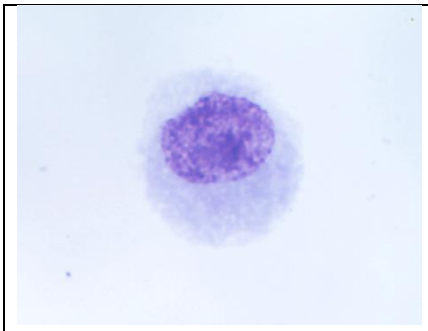
Table 02: Showing Micronucleus Frequency **Vs** Nuclear Division Index results of CBMN assay.

GROUP	Micronucleus Frequency	Nuclear Division Index
Normal	0.014	1.66
Oral Cancer	0.298	2.26

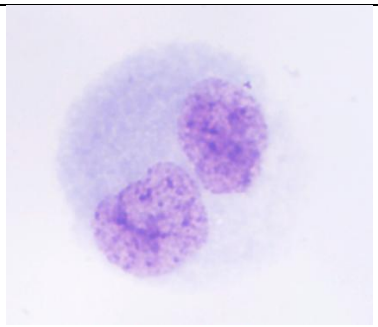


Graph: 03

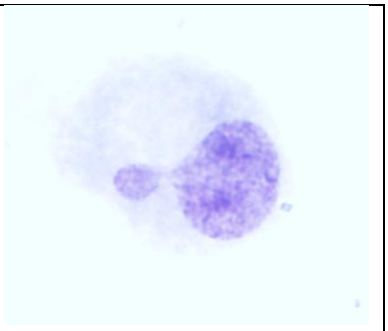
Showing Micronucleus Frequency **Vs** Nuclear Division Index results of CBMN assay.



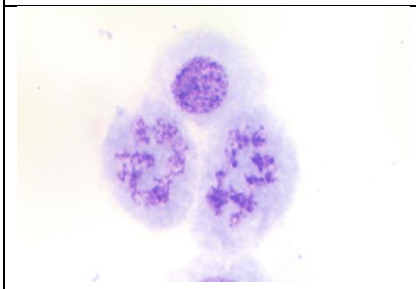
Mononucleated Cell



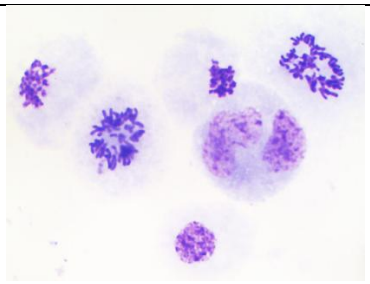
Binucleated Cell

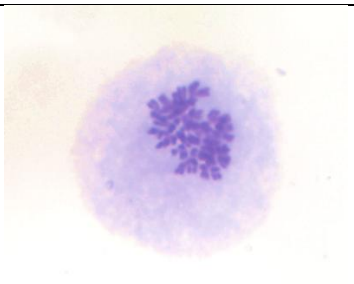


Mononucleated Bud

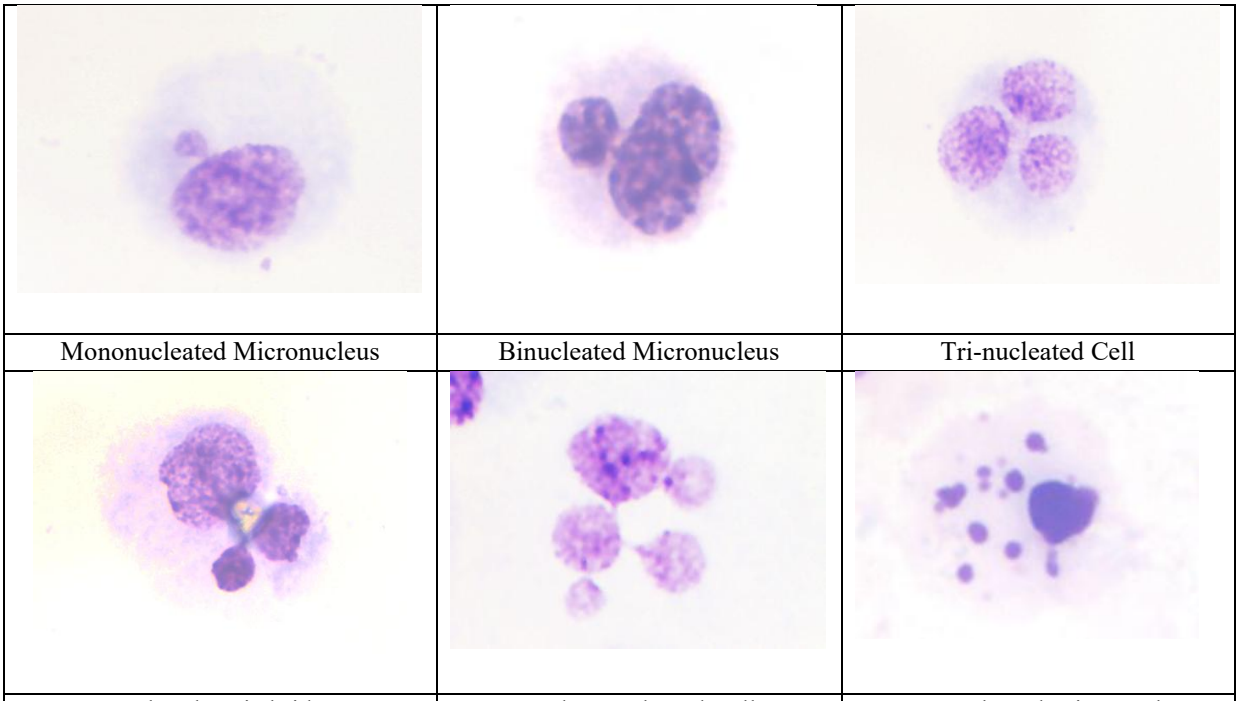


Necrotic Cell





Cells with Polarized nucleus



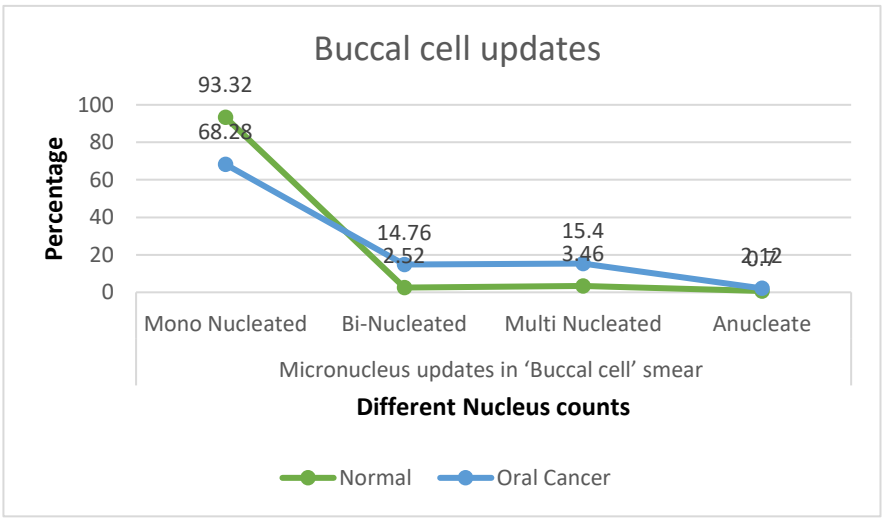
Mononucleated Micronucleus	Binucleated Micronucleus	Tri-nucleated Cell
Nucleoplasmic bridge	Qudra-Nucleated Cell	Mononucleated Micronucleus

Photograph: 01
Showing different cellular alterations after (*in vitro*) CBMN assay of Oral cancer patients' blood samples. Giemsa stain images taken by Motic- BA210, 100X Microscope.

Table: 03 Showing comparative updates of Micronucleus in 'Buccal cell' smear.

Group	Micronucleus updates in 'Buccal cell' smear			
	Mono Nucleated	**Bi-Nucleated**	**Multi Nucleated**	**Anucleate**
Normal	93.32 ±0.476	2.52 ±0.4	3.46 ±0.366	0.7 ±0.223
Oral Cancer	68.28 ± 0.847	14.76 ± 0.750	15.4 ± 565	2.12 ± 0.291

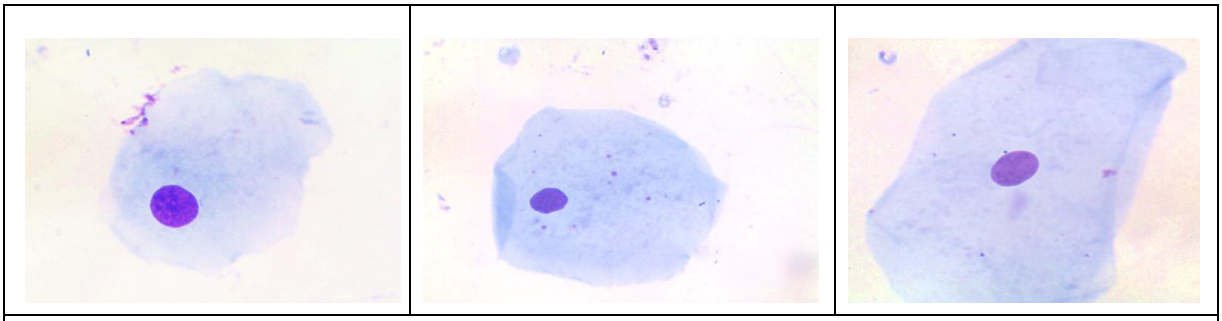
P Value = **0.045**, Significant level (<0.05)



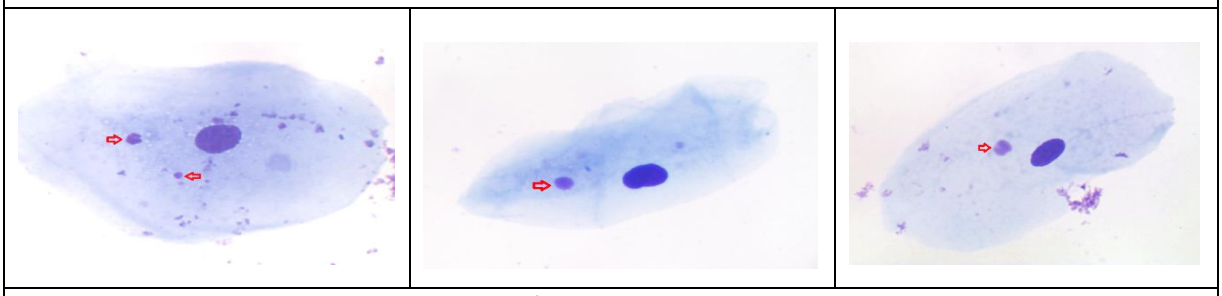
Graph: 04 Micronucleus updates in Buccal cell counts.

Table: 04 Showing Micronucleus Frequency **VS** Nuclear Division Index results of Buccal cell smear (BCMN).

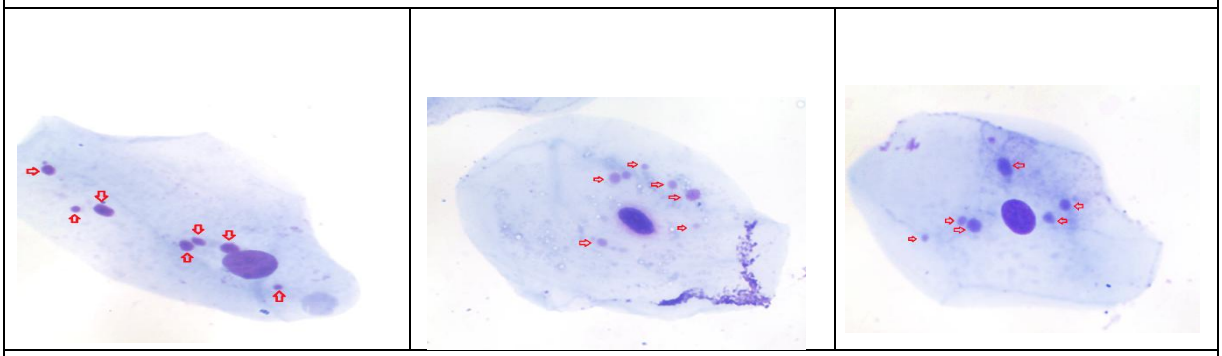
GROUP	Micronucleus Frequency Percentage %	Nuclear Division Index
Normal	6	1.087
Oral Cancer	30.16	1.44



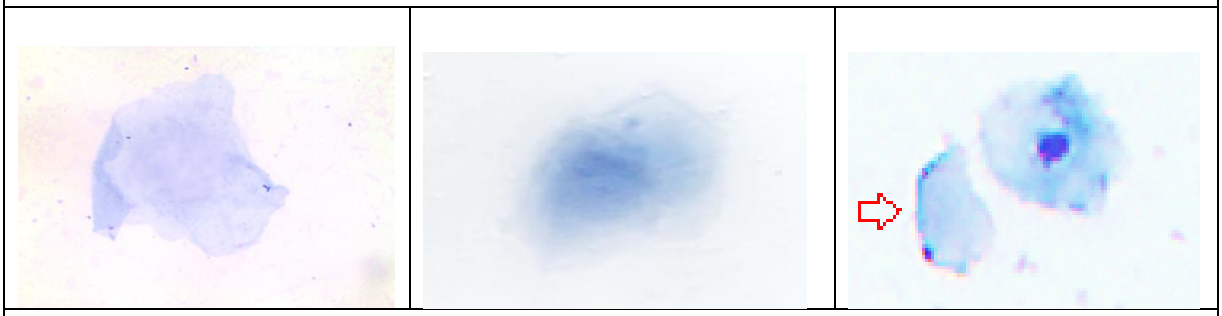
Mononucleated cells (Normal)



Binucleated cells



Multi-nucleated cells



Cells without nucleus (Anucleate)

Photograph: 02

Showing 'Oral Buccal cell' of 'Oral cancer patients' with Micronucleus
Giemsa stain images taken by Motic- BA210, 100X Microscope.

IV. RESULT

Buccal cell micronuclei (BCMN):

The study included 25 oral cancer subjects with an average age of **54.12** years & 10 Normal individuals of an average age of 25 years (normal allocation, standard deviation 10.01 years). With mix population of male and female individuals. Characterizes the total group in terms of the major independent variables like (Table:4 and 5)age, sex, ongoing treatment and oral cancer status. The average percentage of micronucleus for the total population was **14.76** (Binucleated), **15.4** (Multinucleated) micronuclei per 1000 cells counted from 'Buccal mucosal' smear of Oral cancer subjects as compare to Normal subjects (Negative control) micronucleus rate of **2.5**(Binucleated), **3.5** (Multinucleated) per 1000 cells (Table: 10 and 11). The percent of Micronucleus was five to seven times higher in patients with 'Oral cancer' as compared to normal in the control group and the difference was found to be highly significant a graphical presentation (Bar & Liner) is given in (Graph: 4,5,6 and 7). Buccal cell micronuclei (**BCMN**) are considered a non-invasive biomarker for oral cancer detection, as they can be detected in exfoliated Buccal cells. Elevated levels of micronuclei in Buccal mucosal cells are associated with precancerous lesions and oral cancer, making the BCMN assay a useful tool for early detection.

Cytokinesis-Block Micronucleus (CBMN) assay:

Therefore, cytogenetic injury seems to be a superior biomarker for finding the influence of exposure to chromosome harming agents in smoke. Cytogenetic damage can be quantitatively measured by cytogenetic biomarkers such as Cytokinesis-block micronucleus (CBMN) assay is one of the benchmark cytogenetic tests for measuring chromosomal damage in PBLs because of its simplicity, good duplicability, and dependability. We did not come across any study which estimated the level of MN in peripheral blood of oral cancer subjects by CBMN assay. Thus, the present study was conducted to find out whether there is pronounced contrast in genotoxicity in oral cancer patients and healthy individuals by CBMN assay in human peripheral blood lymphocytes by evaluating the average number of MN.

In our study, a novel attempt was made to correlate BCMN and CBMN with MN. We noted a strong significant positive correlation between these two parameters. MN frequency was attributed to cancer stage and age is also an important factor and thus by making the use of this comparative study, we were able to predict the MN frequency in PBMC & BCMN from oral cancer subjects. In contrast, these two parameters showed significant increase in micronucleus formation in diseased subjects. Our results regarding 'Micronucleus Frequency' & 'Nuclear Division Index' shows the cytotoxicity or DNA damage. Likewise, the higher micronucleus frequency observed in oral cancer subjects 0.298 as compare to healthy individual 0.014 in CBMN results. In contrast 2.26 and 1.66'Nuclear Division Index' values observed respectively shows an increase both MF & NDI shown in (Table: 6 and 7) and the graphical representation of data in (Graph: 1,2 and 3) after comparison with normal group.

V. DISCUSSION &CONCLUSION

Detection of micronuclei and their assay is an upcoming research domain in the field of cancer prevention and therapeutics. The presence and frequency of MN represent genomic damage. These miniature nuclear offshoots, if properly identified can turn out to be important biomarkers with huge potential in screening and predicting patients with oral potentially malignant disorders and also can act as risk assessors in patient's ongoing treatment for invasive cancers. The frequency of increase in MNC's from normal mucosa to potentially malignant disorders to oral cancer can suggest a link of this biomarker with malignant neoplastic progression. Therefore, MN assay in exfoliated & Blood cells holds promise as a specific biomarker for exposure to various carcinogens, and can also be used as a screening test in oral health centres. The advantage of micronuclei assay lies in its simplicity as scoring of MN is rapid, practical, and does not require much expertise. It can be concluded from this investigation that besides factors such as alcohol and tobacco, subgingival plaque might be of genotoxic relevance. There was a statistically pronounced increase in the MN frequency in Oral cancer subjects when compared to healthy individuals. MN number also had a correlation with age of individuals.

Our results deduced the fact that the genotoxicity in Oral cancer was profoundly higher by comparison to that of (Healthy) normal group. The results deduced the fact that we can predict the Micronuclei frequency in an individual by obtaining the Buccal sample & Blood sample to compare the seriousness of condition or to check the DNA damage level. This in turn would allow us to evaluate the genotoxicity in oral cancer suspects at an initial stage even before the clinical signs of cancer develop. This would further help in conducting screening programs among high-risk individuals for cancer with family history and consumption of tobacco or other harmful things like alcohol etc. (Casartelli*et al.,* 2000) concluded that the gradual increase in MN counts from

normal mucosal toprecancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression. The present study has revealed that there is a correlation of significant increased frequency of micronucleus present in Oral cancer subjects as compared to normal counterparts, indicating strong cytogenetic damage which may lead to cancerous proliferation.

VI. FUTURE PROSPECTS

A higher sample size with longer duration follow-up is the need to further verify the correlation between the number of MN in peripheral blood lymphocytes & Buccal mucosal cells and its use in cancer risk prediction in individuals. Oral carcinogenesis is a multistep process of accumulated genetic damages leading to cell dysregulation with disruption in cell signalling, DNA-repair, and cell cycle events, which are fundamental to hemostasis. These events can be conveniently studied in the buccal mucosa, which is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects (Jois HS. et al.,2010).The present micronucleus study based on CBMN &BCMN shows a feasible and economical method which could be used as a screening test in population having the family or health history of habits like alcohol and smoking or betel nut chewing for identifying the effects of genomic instabilities and to introduce timely interventional strategy in order to treat and control the epidemic through these low cost strategies and methods aimed at reducing the expense of diagnosis without significantly compromising its quality or effectiveness. These two techniques can be applied to make the oral cancer diagnosis more accessible and affordable and act as biomarker and a tool for pre diagnosis of disease like cancer.

Funding:
"All authors declare that no funds, grants, or other support were received during the preparation of this manuscript and research work done".

Competing Interests:
"The authors have no relevant financial or non-financial interests to disclose."

Author Contributions:
"First authors [Priyanshu Singh] contributed to the study experimental part. Material preparation, data collection and analysis were performed by [Sarfaraz Hanfi, Sameena Akhter & Alibha Guru Rawat]. [Shweta Azad & Rachna Jain] permit to arrange sample from Department of Pathology, JNCH & RC. The first draft of the manuscript was written by [Sarfaraz Hanfi & Priyanshu Singh] checked by [Ankur Chhari, Shivani Tomar and Shivani Deshmukh] all authors commented on previous versions of the manuscript all authors read and approved the final manuscript."

Ethics approval:
"This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Jawaharlal Nehru Cancer Hospital, Idgah Hills, Bhopal, (M.P) India. (Date 20/12/2024, Approval No. 1130 h/JNCH/RES/20/12/24)."

Consent to participate:
"Informed consent was obtained from all individual participants only their Health history &oral swab sample was used in the study."

Consent to publish:
No photographs and images of any participants are added in the submitted manuscript.

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