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RESEARCH ARTICLE

Prevalence of Micronucleated Cell in Buccal Smears among Smokers and Non-smokers

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Abstract

Tobacco addiction is responsible for the huge increase in diseases such as cancer and heart problems. One of the major constituents of environmental toxins is tobacco smoke which is responsible for deaths throughout the world. The process of aberrant mitosis gives rise to micronucleus. Micronucleus is a cytoplasmic chromatin mass which is either oval or round in shape and is visible through a microscope. The buccal cell MN assay has generated enough international interest which has caused the Human Micronucleus project (HUMN) to initiate a new project to validate it. The oral tissues suffer severe damage due to the oral smoking of tobacco on a continuous basis. Smears are a pivotal method for the determination of the damage caused to the oral tissues due to tobacco smoke. Smears help detect the micronuclei in the exfoliated cells of the oral tissue to determine the damage. Our study helped determine the direct impact of tobacco smoke on the cytogen (micronuclei). The study examined 100 individuals who included active, passive and non-smokers. F-test was used as a statistical tool for analysis. The analysis showed that smokers had a higher count of micronuclei per 100 cell counts in comparison to the non-smokers. The results signified a direct relation between the existence of number of micronuclei to that of the tobacco habits of the smokers including the frequency and duration of smoking. Besides, the study also showed an apparent increase in the MN count in the middle age segment of the participants.

INTRODUCTION

The health complexities caused due to tobacco smoking has not been restricted to any geographic region and has spread world-wide (Chandirasekar, R. *et. al*. 2014). The harmful effects of tobacco smoking have acquired global reach now and are no longer limited to only developed countries. The spread of tobacco smoking habits to developing nations has led to an increase in smoking related diseases and even deaths throughout the world (Peto, R. 1996). The number of smokers has increased substantially world-wide and is predicted to reach 1.64 billion in 2025 from its current number of 1.25 billion. Moreover, the increase is accompanied by a simultaneous increase in smoking related deaths per year which is predicted to reach ~10 million per year in a span of 30–40 years' time from its current number of ~3 million deaths per year (Gavin, A. 2004). The estimates presents a disturbing scenario which predicts that almost half billion people of the current world population will die due to diseases related to tobacco (DeMarini, D. M. 2004).

Indoor smoking is one of the major contributors of pollutants in the environment where it is not prohibited. The presence of tobacco smoke in the environment or second hand smoke comprises numerous harmful constituents which pose a serious threat to the people's health (Avsar, A. *et.al.* 2008 and Okamoto, T. *et.al.* 2014). Numerous research studies accompanied by relevant evidence have corroborated the fact that passive smoking is carcinogenic

to people who are non-smokers and suffer the harmful effects due to exposure to tobacco smokes in the environment (Husgafvel-Pursiainen, K. 2004). Smoking has been declared to be one of the major contributors to health hazards like cancer, cardiovascular diseases and chronic obstructive pulmonary diseases by the International Agency for Research on Cancer (Zhang, Y. *et.al*. 2015 and Chandirasekar, R. *et. al*. 2011). Cigarette smoke comprises approximately 4500 chemical compounds including mutagens. The human genetic material suffers irreversible damage due to the mutagens present in the cigarette smoke. Cigarette smoke is thus one of the major mutagenic factors which cause severe damage to the human genetic material (Chandirasekar, R. *et. al*. 2011). The harmful impact of cigarette smoke on the genetic material of active and passive smokers was determined by the following investigation.

Some of the prominent cytotoxic substances present in a cigarette are polycyclic aromatics hydrocarbons, nitrosamines, aromatics amines, heavy metals, pesticide residues and poisonous gases (Umadevi, B. *et. al.* 2003). However, nicotine has been determined to be the major addictive substance present in the cigarettes. The pH of the cigarette smoke helps determine whether nicotine is present in ionized or non-ionized form. Nicotine is found to be present in non-ionized form in alkaline smokes of cigars and pipe tobacco which is absorbed orally in the human body (Backinger, C. L. *et. al*. 2008, Kamath, V. V. *et. al.* 2015). Electron Spin Resonance spectroscopy has estimated the presence of 1 × 10^17 radicals/g of tar or 4 × 10^4 per puff in the cigarette smoke. Tar has been determined to be the most harmful constituent of the tobacco smoke and is a particulate matter which is obtained by the condensation of the cigarette smoke (Kamath, V. V. *et. al.* 2015).

The process of aberrant mitosis gives rise to the micronucleus (MN). Micronucleus is a round or an oval chromatin mass which is visible through a microscope. The chromatin mass is present in the extra vicinity of the nucleus and comprises eccentric chromosomes, chromatid fragments or whole chromosomes, which failed to reach the spindle poles during the process of mitosis (Holland, N. *et.al*. 2008, and Cheng, T. *et. al*. 1996). The micronucleus serves an important role as a biomarker for the assessment of damage in DNA of the affected individual (Cheng, T. *et. al*. 1996).

This study helps identify and correlate the presence of MN in the epithelial cells of active smokers, non-smokers and passive smokers.

Material and methods

A wooden tongue depressor is used for the collection of buccal mucosa of the participants. A sample of buccal mucosa can be collected either via swabbing or by the scrapping of the mucosa from the inner linings of the cheeks. The collected cells are then spread on a clean slide to create a smear which is then stained with methylene blue. The smears are then observed under 100 magnifications. The process of identification of micronuclei involves the counting of 100 cells per slide.

The subjects are then divided into three groups; smokers (N=100), non-smokers (N=100) and passive smokers (N=100), each of which comprises 100 members. The variable 'N' represents individuals who are aged 25 years or more. The study involved only male smokers which relegated the need to balance sex bias. A standard questionnaire was used to collect personal information of the participants such as their age, smoking habits, health status and medications.

Result

The study involved the counting of 100 buccal cells of each of the participants. The presence of micronuclei in each of these cells was further noted to help determine the pattern of existence of micronuclei in the cells of smokers, non-smokers and passive smokers [Figure 1, NON-SMOKER WITHOUT MN]. It was found that micronuclei were either single or multiple in numbers in the analyzed buccal cells and were found to be more prominent in the buccal cells of the smokers [Figure 2 smokers with 2 or more MN]. The studied showed that the MN count was higher in buccal cells of smokers in comparison to that of the non-smokers and passive smokers [figure 3 with 1 MN]. The results were in conformance with the statistical study of variance analysis *f*-test ($P < 0.000$) [Table 1].

Moreover, the analysis of the results also showed the relation of the MN count to that of the age of the participants. The MN count of the group comprising members <25 years was found to be less than the MN count of the group comprising members >25 years and <50 years. However, the group comprising participants >50 years showed MN count which was the highest among the three groups. The P value was found to be significant statistically [Table 2].

The study also studied the correlation between the MN counts per 100 cells with the duration of smoking. It was found that MN count was higher in groups with participants who smoked for > 12 years as compared to the group who smoked for time between 6 and 12 years and < 6 years [Table 3]. The MN count was also correlated with the

frequency of smoking in the participants. The study showed that MN count per 100 cells were higher in groups comprising members who smoked >12 cigarettes per day in comparison to those who smoked between 6 and 12 cigarettes per day and <6 cigarettes per day [Table 4].

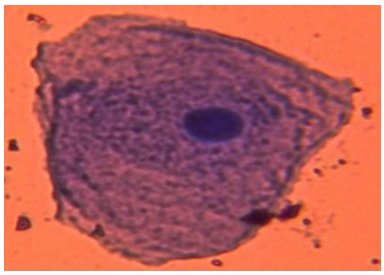


Figure 1 Non-Smokers without MN

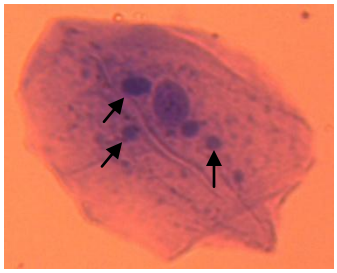


Figure 2 Smokers with two or more MN

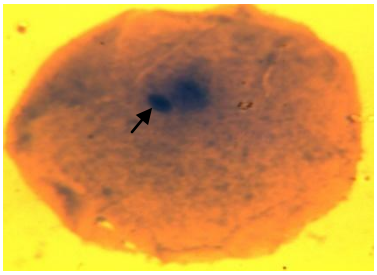


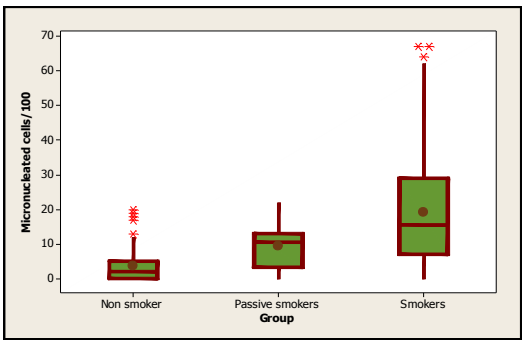
Figure 3 Passive smokers with one MN

Table 1: Statistical analysis of micronuclei in smokers, passive smokers and non-smoker

Group	Mean	**Standard deviation**	F	P value
Non smoker	3.61	4.76	56.34	0.000
P-smokers	9.43	6.44		
Smokers	19.31	16.47		

Table 2: Micronuclei count in relation to age in smokers

Group	Mean	**Standard deviation**	F	P value
<25	5.57	7.06	24.69	0.000
25-50	9.42	10.41		
>50	18.49	16.10		



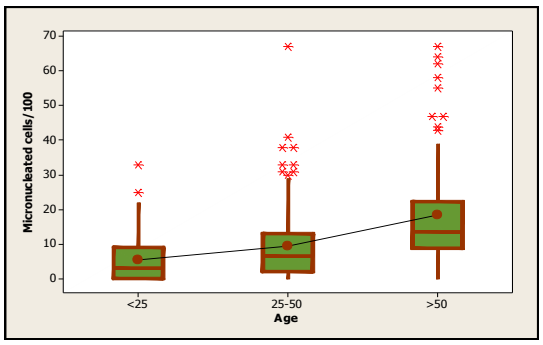
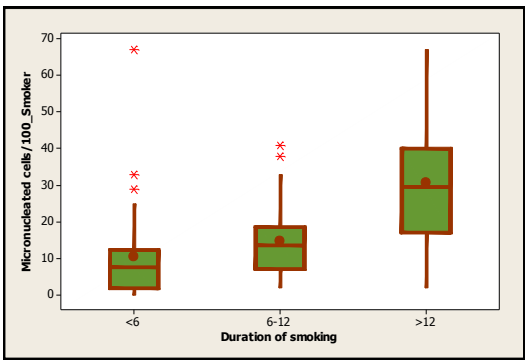


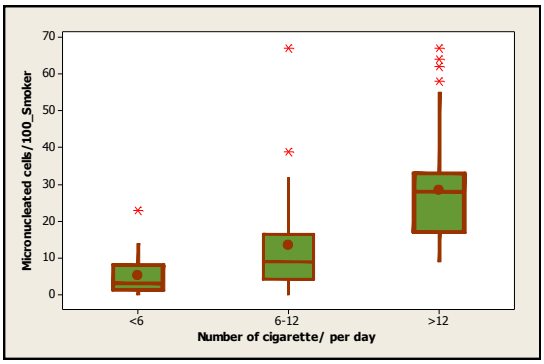
Table 3: Micronucleated cells/100 Smoker versus Duration of smoking.

Duration of smoking (years)	*N*=Total members in the group	Mean	Standard deviation	F	P value
<6	34	10.44	13.23	21.56	0.000
6 – 12	28	14.57	10.46		
>12	38	30.74	16.33		

Table 4 Micronuclei in relation to frequency of smoking.

Frequency of smoking (cigarettes/day)	*N*=Total members in the group	Mean	Standard deviation	F	P value
<6	24	5.7	5.51	29.01	0.000
6-12	24	13.46	14.77		
>12	52	28.54	14.75		





Discussion

An increased chromosome breakage in micronuclei (MN) leads to higher cancer risks (Sasikala, K. 2011). A recent study has validated the aspect of association of frequent chromosomal aberrations with higher cancer risks in individuals (Proia, N.K. *et.al*. 2006). Micronuclei are described as chromosomal fragments or whole chromosomes which are removed from the vicinity of nucleus during the process of mitosis (Ceppi. M. *et.al* 2010). The scoring of MN in buccal cells is a fast and simple process unlike the scoring of chromosomal aberrations. However, an evaluation of the genotoxic consequences of environmental and occupational mutagens and carcinogens on the human health was done by MN assay (Cheng, T *et.al.* 1996).

The study focussed on assessment and analysis of MN count among the smokers, non-smokers and passive smokers to help determine the group which was at an increased risk of cancer. The results showed that the MN count was higher in the group comprising smokers as compared to that of non-smokers and passive smokers. The result corroborates the genotoxic effect of tobacco smoke on the smokers (Kamath, *et.al*. 2015) as the MN count was found to be double in smokers as compared to non-smokers with high statistical significance and was thus in conformance with the results of this study.

The results also showed that the MN count was higher in the group with participants >50 years. The genotoxic effect of tobacco smoke increased on the mucosal lining of the cells over long periods of time which caused an increase in the MN count. The effects of genotoxic substances such as tobacco and radiations are not limited to the affected cells as it causes DNA damage which are then passed on to the daughter cells (Holland, N. *et.al.* 2008). MN reflects the damages suffered in the DNA which may lead to pre-cancer or cancer. As a result, assessment of MN count along with a high index would help determine the tendency of the individual to develop such detrimental changes in the future. The knowledge to help predict the probability of the individual to develop such harmful consequences in the future due to smoking habits can be used as an effective screening and educational medium. The knowledge can be used to monitor and educate the individuals in giving up the harmful habit of smoking. However, longitudinal studies need to be done to help in the quantification and support of the aforementioned contention.

Furthermore, the study showed interesting results in the MN count of participants on the basis of duration of their smoking habits. MN count was found to be higher in individuals with >12 years of smoking in comparison to individuals with >6 years and <12 years and <6 years of smoking. A significant difference in MN count was found among them ((P value = 000). The considerable difference can be explained by the fact that nucleus regulates MN, which is a cytological feature. Moreover, results also showed MN counts to be higher in participants who smoked >12 cigarettes/day in comparison to participants who smoked between 6-12 cigarettes/day and <6 cigarettes/ day. The difference was due to increased genotoxic effect of the tobacco smoke and environmental factors on the oral mucosa of the smokers with higher frequency of smoking per day.

The aspect of chromosomal breakage in the early process of cell divisions and the number of increased micronuclei is determined by the frequency of the existence of micronuclei. The frequency of existence of micronuclei is accelerated by the presence of carcinogenic stimuli and is an effective determinant as the manifestation of clinical symptoms for the above diagnosis takes time (Kapka, *et.al*. 2007 and Kamath, *et.al*. 2015). MN assay is a simple process which involves rapid scoring of MN and does not require expertise to conduct it. MN can be called as an "internal dosimeter" of the cell which helps in determining the harm caused to it by the genotoxic and carcinogenic substances (Kamath, *et.al.* 2015 and El-Zein, R. *et.al*. 2011).

Conclusion

Epithelial tissues are apt for determination of any harmful changes in the body due to smoking. The epithelial cells are directly influenced by the effects of genotoxic impacts of tobacco smoking. Besides, about 90% of cancers are ascertained to exist in the epithelial tissues which make their collection as samples necessary and easy as it does not cause any discomfort to the patients. The laboratory process involving epithelial tissues as sample is simple, cheap, accurate and quick. Micronuclei are whole chromosomes or chromosomal fragments which are released from the nucleus during the process of mitosis. Moreover, scoring of MN in buccal cells is preferred over chromosomal aberrations as it is both simple and quicker. Additionally, MN assay helps in an accurate estimation of the gentoxic effects of the mutagens and carcinogens present in the environment on the affected individuals' health.

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