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ORIGINAL RESEARCH

Comparison of micronucleus assay in smokers and non smokers employing feulgen stain

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Abstract

Background: Smoking tobacco can seriously harm general health and increase the risk of cancer. Micronuclei are entities that develop in cells of buccal mucosa following genomic damage, and they may be a helpful predictor of chromosomal changes in maintaining cytological data. These cells also exhibit the nuclear abnormalities that result from cell damage.

Aim: To evaluate effect of fuelgen staining procedures on the results of micronucleus assay in the exfoliated buccal mucosal cells of smokers and non-smokers.

Methods and materials:A total of 100 subjects were examined in the study, which were divided into 2 groups. Each group had 50 subjects. Group1. Comprised of the control group which included tobacco non user's individuals i.e. those who are without any habit and Group 2 included tobacco smokers.This study was performed to observe and calculate MN index in oral epithelial cells and to compare MN index within above mentioned groups. Feulgen stain (counter stained with fast green), was used to code and stain the slides.

Results: Maximum mean MN count per 500 cells were in group –2 (76.14 $\pm$ 18.39) followed by group – 1 (28.96 $\pm$11.20).One way ANOVA test found that mean MN count per 500 cells were not alike in both groups. Post Hoc Turkey HSD Test found in pair wise comparison that there was statistically significant difference ($p<0.05$), between group 1 and group 2.

Conclusion: When correlation between various smoking or tobacco use indicators and MN count was evaluated, significant positive correlation with all parameters was observed

Keywords: Micronuclei, smokers, non smokers.

Introduction

Many studies have been conducted recently to try and understand how genetic (deoxyribonucleic acid (DNA)) detrimental lifestyle factors like smoking, drinking, eating a diet low in vitamins and needing supplements, stress, and drug use affect human populations.

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Using cytochemical techniques such as the micronucleus (MN) assay, sister chromatid exchange, and chromosomal abnormality, this DNA damage can be examined. Since its initial introduction in 1983, the micronuclei assay has grown in prominence as a predictor of cellular mutations for the identification of elevated cancer risk.[1]

It is widely used since it is a quick, easy, affordable, and noninvasive approach. As a result of clastogenic (which results in chromosomal breakage) and aneugenic (which results in aneuploidy or aberrant chromosome segregation) events, MN contains genetic material that is lost during mitosis. . This test is useful for determining the degree of DNA damage in a cell and provides details about a person's risk of developing cancer. Tolbert et al. published two studies in 1991 and 1992 that proposed a new standard for cell counts in MN tests.[2]

Degenerative nuclear phenomena, in his opinion, ought to be counted separately during MN analysis because a greater prevalence of this phenomenon alone is indicative of apoptosis phenomenon (karyorrhexis (KH), condition of condensed chromatin (CC), and condition of pyknosis (PN), condition of necrosis which disclose the genotoxic as well as cytotoxic consequences, respectively.[3]

Thomas et al. (2009) state that I cells with MN and nuclear buds evaluate DNA damage, (ii) cells with KH, KL, P, and CC draw conclusions about instances of cell death, (iii) basal cells evaluate the mucosa's proliferative potential, and (iv) binucleates cells reveal information on cytokinesis defects. Before any systemic problem manifests, oral mucosal buccal cells are the cells that are first exposed to several potentially carcinogenic chemicals, such as alcohol and tobacco. As a result, these cells are vulnerable to damage from these agents.These cells, which have a high rate of proliferation, represent the root of >90% of epithelial cancer cases.[4]

As a result, it is thought that using this test on epithelial cells is a sensitive way of testing the genetic mutations in the population of humans. More important in preventing and lowering the number of fatalities is screening people who are more likely to develop cancer than the pricey and invasive treatment given later. In order to prevent any false positive tests, it is necessary to accurately count these cells (micronuclei + nuclear abnormalities) using particular staining techniques. The impact of various staining techniques on the outcomes of the recently popularised micronuclei assays has received very little consideration up until this point.[5] There aren't many research that evaluate micronucleus assay in the exfoliated buccal mucosal cells of smokers and non-smokers. Therefore this study was carried out to analyse the effect of staining procedures involving fuelgen stains on the results of micronucleus assay in the exfoliated buccal mucosal cells of smokers and non-smokers.

Methods and Materials

A total of 100 subjects were examined in the study, which were divided into 2 groups. Each group had 50 subjects. Group1. Comprised of the control group which included tobacco non user's individuals i.e. those who are without any habit and Group 2 included tobacco smokers. The average daily cigarette consumption for all smokers was one pack (10 cigarettes per pack). None of the subjects had a history of systemic illness, drug use, or radiation exposure, and they were all in good condition. All participants provided their informed permission, and the institution granted ethical clearance. Before setting up the cytological smears, subjects were instructed to give their mouths two thorough rinses with water.

Slide was created from each subject by gently scraping the exfoliated buccal mucosal cells over the cleaned glass slides with a wooden spatula.Feulgen stain (counter stained with fast green) was used to code and stain the slides. 10 microscopic fields from each smear were examined at magnifications of 10 and 40. (light microscope was used). Buccal cells were graded for micronuclei using the standards established by Tolbert et al. According to Thomas

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et al., the same nuclear anomalies, including binucleation, CC, KH, KL, PN, and nuclear bud, were also detected in Feulgen stain.

Statistical analysis

To compare the differences in micronuclei between smokers and nonsmokers, two-way analysis of variance (ANOVA) was utilised. A p value ≤0.05 was considered statistically significant.

Results

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Table 1 shows that maximum mean MN count per 500 cells were in group –2 (76.14 ± 18.39) followed by group – 1 (28.96 ±11.20).One way ANOVA test found that mean MN count per 500 cells were not alike in both groups. Post Hoc Turkey HSD Test found in pair wise comparison that there was statistically significant difference (p<0.05), between group 1 and group 2.

On performing intra group analysis, it was found that among subjects of group-2, 86% were cigarette smokers, while 14% were bidi smokers. All 86% cigarette smokers were using filtered cigarettes. Similarly all 14% bidi smokers were considered to be using non filtered smoke. (Table 3).

As stated in table 4, Mean MN count per 500 cells among bidi smokers were (91.57± 16.15) which was higher than that in case of cigarette smokers (73.63±17.64). Unpaired t- test found that bidi smokers have significantly higher MN count per 500 cells than in case of cigarette smokers (p>0.05).

Above table shows that average duration of habit of smoking was 7.56±5.19. Similarly 9.70±7.68 cigarettes per day was an average quantity used by a smoker in group 2 which contains 5.77±2.99 tobacco quantity per day on an average. When this is expolated on yearly consumption of tobacco, it was 2105.62±1091.89.

When correlation between various smoking or tobacco use indicators and MN count per 500 cells was calculated, significant positive correlation with all parameters was observed (p <0.05). Average tobacco quantity per day and average yearly consumption of tobacco were most correlating indicators with values of 0.7316 and 0.7317 respectively. (Table 5)

Table-1: Comparison of study groups w.r.t. MN count per 500 cells.

Group	N	Mean	SD	Median	Min.	Max.	95% CI	'p' Value*	Significant Difference From**
Non-tobacco Users (1)	50	28.96	11.20	26	15	55	25.777-32.143	<0.001	2,3
Smokers (2)	50	76.14	18.39	75	50	115	70.914-81.366		1,3

* ANOVA - Analysis of Variance **Post hoc Tukey HSD Test

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Table 2: Distribution of smokers (group 2) according to type of smoking.

Type of Smoking	No.	%
Bidi	7	14.00
Cigarette	43	86.00
Total	50	100.00

Type of Smoking	No.	%
Filtered	43	86.00
Non-Filtered	7	14.00
Total	50	100.00

Table 3: Comparison of type of tobacco with MN count/500 cells within group 2.

Tobacco type	N	Mean	SD	Median	Min.	Max.	95% CI	'p' Value*
Bidi	7	91.57	16.15	85	75	113	76.631 - 106.511	0.015
Cigarette	43	73.63	17.64	68	50	115	68.200 - 79.056	

***Unpaired 't' test**

Table 4: Habit indicators of smokers.

	Mean	SD
Average quantity (no.)/day	9.70	7.68
Average yearly Consumption of tobacco	2105.62	1091.89
Average Tobacco Qt/day	5.77	2.99
duration of habit(yrs)	7.56	5.19
Average Cumulative Consumption of tobacco	19085.75	24042.27

Table 5: Correlation of MN per 500 cells with various habit indicators of smokers.

	N	Correlation coefficient r	'p' Value	95% Confidence interval for r
Average Cumulative Consumption of tobacco	50	0.7211	<0.0001	0.5540 to 0.8324
Average quantity (no.)/day	50	0.6644	<0.0001	0.4737 to 0.7956
Average Tobacco Qt/day	50	0.7316	<0.0001	0.5692 to 0.8391
Average yearly Consumption of tobacco	50	0.7317	<0.0001	0.5693 to 0.8392
duration of habit(yrs)	50	0.6959	<0.0001	0.5178 to 0.8161

Discussion

It was observed in our study that maximum mean MN count per 500 cells were in group –2 (76.14 ± 18.39) followed by group – 1 (28.96 ±11.20).One way ANOVA test found that mean MN count per 500 cells were not alike in both groups. Post Hoc Turkey HSD Test found in pair wise comparison that there was statistically significant difference ($p<0.05$), between group 1 and group 2.

On performing intra group analysis, it was found that among subjects of group-2, 86% were

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cigarette smokers, while 14% were bidi smokers. All 86% cigarette smokers were using filtered cigarettes. Similarly all 14% bidi smokers were considered to be using non filtered smoke.Unpaired t- test found that bidi smokers have significantly higher MN count per 500 cells than in case of cigarette smokers ($p>0.05$).

When correlation between various smoking or tobacco use indicators and MN count per 500 cells was calculated, significant positive correlation with all parameters was observed ($p <0.05$). Average tobacco quantity per day and average yearly consumption of tobacco were most correlating indicators with values of 0.7316 and 0.7317 respectively

The results of the present investigation showed that the staining techniques had a significant impact on the micronuclei assay in exfoliated oral mucosal cells of smokers and non-smokers.

Numerous studies have been carried out recently to try and understand how harmful lifestyle factors, such as smoking, drinking, eating a diet low in vitamins and needing supplements, stress, and drug use, affect human populations. These factors are genetic affecting deoxyribonucleic acid (DNA). This DNA damage can be examined using cytochemical methods like the micronucleus (MN) assay, sister chromatid exchange, and chromosomal abnormality.[6,7] The micronuclei assay has gained popularity as a predictor of cellular alterations for the diagnosis of higher cancer risk since it was first introduced in 1983.

According to Thomas et al. (2009), basal cells can be used to assess the mucosa's proliferative capability, nuclear buds and MN cells help to assess DNA damage, KH, KL, P, and CC cells are indications of cell death, and binucleate cells are indications of cytokinesis abnormalities.[8,9]

Oral mucosal buccal cells are the first cells to be exposed to a number of possibly cancer-causing substances, including alcohol and cigarettes, before any systemic problem appears. These cells are hence susceptible to harm from these substances. More than 90% of instances of epithelial carcinoma are caused by these cells, which proliferate quickly.Because of this, it is believed that applying this test to epithelial cells is a sensitive method of determining the genetic mutations present in the human population.[10]

Screening those who are more likely to get cancer is more crucial to avoiding and reducing the number of fatalities than the expensive and invasive treatment provided later. It is essential to precisely count these cells (micronuclei + nuclear abnormalities) using specific staining procedures in order to avoid any false positive testing. The counting of MN is obscured by the use of the Romonswsky-based Giemsa dye, which stains cell detritus and numerous other proteins. As a result, mean MN showed improved outcomes.

Certain points were taken into account to prevent MN from being incorrectly counted. Giemsa still showed an elevated mean MN, nevertheless. In numerous investigations, the accuracy of many DNA nonspecific stains has also been questioned. Feulgen's strong DNA specificity and acridine orange's clear, translucent appearance, which make MN easy to identify, may be the cause of the lowest count when used together. The benefit of acridine orange is that it improves the clarity of the appearance of different cells; nevertheless, the costlier evaluation using a fluorescent microscope is a drawback.[9] According to the current study, smokers had considerably more metanucleated cells with abnormalities other than micronuclei, such as KH, KL, binucleates, and CC.These anomalies are a result of cell damage, cell death, and mitotic mistakes. Apoptosis is present in several of them (PN, CC, and KL), which may be brought on by DNA damage.[10]the same phenomena, though, is also present in cells that are undergoing necrosis, thus it is not possible to rely on it as a reliable indicator of increased DNA damage and cancer risk. Only a small number of researches with similar anomalies have been reported. All investigations, including ours, found that KL was significantly higher than control groups, although the other abnormalities were introduced

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indistinctly.[11]

Conclusion

When correlation between various smoking or tobacco use indicators and MN count was evaluated, significant positive correlation with all parameters was observed.

References

1. Alexandresceu I, Havarneanu D, Popa D. New approaches in biomonitoring human populations exposed to genotoxic agents: Epithelial cell micronucleus assay. J Prev Med 2006;14:57-5.
2. Kalita H, Boruah DC, Dutta K, Devi R. Genotoxic effect on buccal epithelial cells of betel quid chewers by micronuclei assay. Asian J Exp Biol Sci 2013;4:491-4.
3. Khanna S, Purwar A, Singh NN, Sreedhar G, Singh S, Bhalla S. Cytogenetic biomonitoring of premalignant and malignant oral lesions by micronuclei assessment: A screening evaluation. EurJ Gen Dent 2014;3:46-2.
4. Torres-Bugarín O, Zavala-Cerna MG, Nava A, Flores-García A, Ramos-Ibarra ML. Potential uses, limitations, and basic procedures of micronuclei and nuclear abnormalities in buccal cells. Dis Markers 2014;2014:956835.
5. Jyoti S, Khan S, Afzal M, Naz F, Siddique YH. Evaluation of micronucleus frequency by acridine orange fluorescent staining in bucccal epithelial cells of oral submucosus fibrosis (OSMF) patients. Egypt J Med Hum Genet 2013;14:189-3.
6. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: Methods development. Mutat Res 1992;271:69-77.
7. Thomas P, Holland N, Bolognesi C, Volders MK, Bonassi S, Zeiger E, et al. Buccal micronucleus cytome assay. Nat Protoc 2009;4:825-7.
8. Nersesyan A, Kundi M, Atefie K, Schulte-Hermann R, Knasmüller S. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiol Biomarkers Prev 2006;15:1835-40.
9. Grover S, Mujib A, Jahangirdar A, Telagi N, Kulkarni P. A comparative study for selectivity of micronuclei in oral exfoliated epithelial cells. J Cytol 2012;29:230-5.
10. Casartelli G, Monteghirfo S, De Ferrari M, Bonatti S, Scala M, Toma S, et al. Staining of micronuclei in squamous epithelial cells of human oral mucosa. Anal Quant Cytol Histol 1997;19:475-81.
11. Palaskar S, Jindal C. Evaluation of micronuclei using papanicolaou and may grunwald giemsa stain in individuals with different tobacco habits- A comparative study. J Clin Diagn Res 2010;4:3607-3