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Original Research

Assessment of micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco chewing habit: A histopathologic study

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ABSTRACT:
Background: Detection of morphological and nuclear abnormalities of the cell are the basis of cytological evaluation and for that, micronucleus frequency evaluation in buccal exfoliative cells is a preferable method for cancer risk assessment because it is less technique sensitive, easy and an appreciable biomarker for genomic instability. Hence; under the light of above mentioned data, the present study was undertaken for evaluating micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco chewing habit. **Materials & methods:** A total of 75 subjects were enrolled and were divided into three study groups as follows: Group A: 25 Diabetic with tobacco chewing habit, Group B: 25 Non-diabetic with tobacco chewing habit, and Group C: 25 Controls. The cyto smears was separately stained with PAP and GIEMSA stains. The slides were mounted with cover glass using DPX mountant and were analysed. Average frequency of MN= Total no of MN/ Total no of cells with MN. Recording of all the results was done in Microsoft excel sheet and were analysed by SPSS software. Chi- square test was used for evaluation of level of significance. **Results:** Mean MF frequency per high power field by GIEMSA stain among subjects of group A, Group B and Group C was found to be 1.19, 0.98 and 0.69 respectively. Significant results were obtained while comparing the mean MN frequency per high power field in GIEMSA stain and PAP stain. **Conclusion:** Type-2 diabetic patients with habits of tobacco smoking had significantly higher frequency of Micronuclei as compared to patients with diabetes and control group.
Key words: Buccal mucosa, Exfoliated, Diabetes

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INTRODUCTION

Diabetic complications in target organs arise from chronic elevations of glucose via increased production of Reactive Oxygen Species and Reactive Nitrogen Species and subsequent oxidative stress. Excessive production of oxygen - free radicals through glucose auto-oxidation and non –enzymatic glycation, especially in diabetic patients with poor glycemic control can accelerate oxidative damage to the macromolecules, including DNA damage.[1-3]

Diabetes and hyperglycemia can be sources of DNA damage via the oxidation of DNA bases and sugar–phosphate binding sites .The occurrence of these alterations can result in mutagenic effects and/or DNA replication arrest and may be associated with risk for developing cancer in diabetes mellitus patients.[4]

As oral cancer is directly associated with genomic instability, detection of morphological and nuclear abnormalities of the cell are the basis of cytological evaluation and for that, micronucleus frequency evaluation in buccal exfoliative cells is a preferable method for cancer risk assessment because it is less technique sensitive, easy and an appreciable biomarker for genomic instability. On microscopic examination Micro Nuclei are seen as small chromatin bodies which appear in the cytoplasm from condensation of detached chromosome fragments or by whole chromosome lagging behind the cell division. The micronuclei are round or oval in shape with their diameter ranging between 1/3rd and 1/6th of the main nucleus. MN has same staining intensity and texture as the main nucleus. Most cells with MN will contain only one MN but it is possible to find cells with two or more MN. Base line frequencies for micronucleated cells in the normal buccal mucosa are usually within the 0.5-2.5 MN/1,000 cells range. Cells with multiple MN are rare in healthy subjects but become more common in individuals exposed to radiation or other genotoxic agents13. The presence of MN is indicative of chromosome loss or fragmentation occurring during earlier nuclear division.[4- 6]

Hence; under the light of above mentioned data, the present study was undertaken for evaluating micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco chewing habit.

MATERIALS & METHODS

The proposed prospective study was conducted in the Department of Oral Pathology and Microbiology, Babu Banarasi Das College of Dental Sciences Lucknow, after obtaining clearance from the Institutional Ethical Committee**.**

Exclusion Criteria:

1. Non- cooperative individuals
2. Individuals with history of Occupational carcinogenic exposure
3. Individuals not fulfilling the above inclusion criteria

A total of 75 subjects were enrolled and were divided into three study groups as follows:

- Group A: 25 Diabetic with tobacco chewing habit
- Group B: 25 Non-diabetic with tobacco chewing habit
- Group C: 25 Controls

Complete demographic and clinical details of all the patients were obtained. Subjects have asked to rinse their mouth gently with water. Exfoliated cells from buccal mucosa will be scraped using a slightly moistened cytobrush/wooden spatula**.** The cells were immediately smeared on two proclaimed microscopic slides for each subject. Just prior to drying, the smear will be fixed with commercially available spray fixative for 15 minutes. The cyto smears was separately stained with PAP and GIEMSA stains. The slides were mounted with cover glass using DPX mountant and were analysed. Average frequency of MN= Total no of MN/ Total no of cells with MN. Recording of all the results was done in Microsoft excel sheet and were analysed by SPSS software. Chi-square test was used for evaluation of level of significance.

RESULTS

Mean age of the patients of group A, group B and group C was found to be 46.72 years, 44.64 years and 45.98 years respectively. There were 12 males, 12 males and 13 females in group A, group B and group C respectively. In the present study, mean MF frequency per high power field by GIEMSA stain among subjects of group A, Group B and Group C was found to be 1.19, 0.98 and 0.69 respectively. Significant results were obtained while comparing the mean MN frequency per high power field in GIEMSA stain and PAP stain.

Table 1: Demographic data

Group	Mean age (years)
Group A	46.72
Group B	44.64
Group C	45.98

Table 2: Gender-wise distribution

Gender	Group A	Group B	Group C
Males	13	13	12
Females	12	12	13

Table 3: MN frequency per high power field GIEMSA

Group	Mean	p- value
Group A	1.19	0.010 (Significant)
Group B	0.98	
Group C	0.69	

Table 4: MN frequency per high power field PAP

Group	Mean	p- value
Group A	4.12	0.020 (Significant)
Group B	2.28	
Group C	0.89	

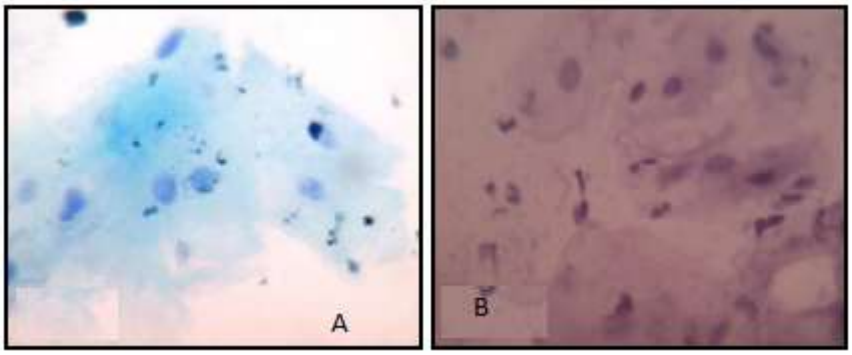

Figure 1: A) Micronuclei in Giemsa stain, B) Micronuclei in PAP Stain

DISCUSSION

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. It is one of the major risk factor accounting for premature mortality and morbidity due to its complications.[7]

India being the diabetic capital of the world has more than 35 million people affected by diabetes currently with future estimation of around 80 million people in 2030. Type 1 insulin-dependent diabetes results from an absolute deficiency of insulin caused by the destruction of insulin-secreting pancreatic β-cells and accounts for only 5–10% of all diabetic patients.[8]

In both Type-1 and Type-2 DM, chronic hyperglycemia is the primary cause of the clinical complications of the disease. Diabetic complications in target organs arise from chronic elevations of glucose via increased production of ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) and subsequent oxidative stress. Excessive production of oxygen-free radicals through glucose auto-oxidation and non-enzymatic glycation, especially in diabetic patients with poor glycemic control, can accelerate oxidative damage to the macromolecules, and cause DNA damage.

Increased Oxidative DNA damage in diabetes may be a useful clinical marker in order to predict its complications. Interestingly, the oxidative DNA damage was higher in individuals with T2D mellitus as compared to those with T1D.[9]

The prevalence of oral cancer was noted to be high in India. Even though it is an established fact that tobacco and related products are one of the leading causative agents for oral cancer, their use is still very prevalent because of its free availability cheaper cost. Smokeless tobacco products including chewing and/or snuffing are believed to face less cancer risk than smoker, but are still at greater risk than people who do not use tobacco products.[10]

India being the diabetic capital of the world, causing clinical complications primarily due to chronic hyperglycemia via increased production of ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) and subsequent oxidative stress. Excessive production of oxygen-free radicals through glucose auto-oxidation and non-enzymatic glycation, especially in diabetic patients with poor glycemic control, can accelerate oxidative damage to the macromolecules, and cause DNA damage. Increased Oxidative DNA damage in diabetes may be a useful clinical marker in order to predict its complications. Interestingly, the oxidative DNA damage was higher in individuals with T2D Mellitus. The results of the present study can be used as a powerful tool for assessing micronuclei by the oral exfoliative cytology, frequency of increased micronuclei can be indicator of risk factors like Cancer. The habits and life style may be an appropriate question to be included in present and past medical history for determination of why some patients of type-2 diabetes are greater risk for oral health than normal.[11, 12]

Jindal and Palasker evaluated micronuclei using papanicolae and MGG stain in individuals with different tobacco habits (smokers, smokeless tobacco and non users). They found micronucleus frequency decreased in smokers than those with other habits and concluded that PAP is a better stain for counting micronuclei when compared to MGG smears where bacteria and cell debris mask the effect of micronucleus.[13]

Lima et al carried out a study to quantitatively evaluate micronuclei in the mucosa of users of alcohol, tobacco and illicit drugs in a Brazilian population and control group. The incidence and frequency of MN were more in the habit related group as compared to the controls. They concluded the MN as an important cytogenic marker in biomonitering habit related individuals.[14]

CONCLUSION

Under the light of above obtained data, the authors conclude that Type-2 diabetic patients with habits of tobacco smoking had significantly higher frequency of Micronuclei as compared to patients with diabetes and control group. However; further studies are recommended.

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