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ORIGINAL RESEARCH

Analysis of micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco smoking habit: An observational study

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ABSTRACT:
Background: Tobacco used in any form can result in irreparable genetic damage and is the most common chemical carcinogen for oral cancer. Diabetes mellitus is a growing and massively silent epidemic that has the potential to cripple health services in all parts of the world. Hence; under the light of above mentioned data, the present study was undertaken for assessing micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco smoking habit. **Materials & methods:** A total of 75 subjects were enrolled and were divided into three study groups as follows: Group 1: 25 Diabetic with tobacco smoking habit, Group 2: 25 Non-diabetic with tobacco smoking habit, and Group 3: 25 Controls. 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. 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. All the results were summarized in Microsoft excel sheet and were analysed by SPSS software. **Results:** The mean MN frequency PER HPF GIEMSA increase with increase in disease severity (normal to type-2 diabetic) and tobacco use. Comparing the mean MN frequency PER HPF GIEMSA of three groups, ANOVA showed significantly different MN frequency PER HPF GIEMSA among the groups. **Conclusion:** Type-2 DM patients have significantly more genetic damage (in terms of MN frequency). This indicates than MN may be a useful constituent in a panel of biomarkers for the risk of diabetes.
Key words: Buccal, Exfoliated, Diabetes.

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INTRODUCTION

The cell is the functional and structural unit of life. The development of multicellular organisms requires a continual source of differentiating cells to populate fields to increase tissues, but defects in this process can lead to developmental abnormalities as well as cancerous growth.[1-3]

Carcinogenesis is a multistep process characterized by genetic, epigenetic, and phenotypic changes. Such changes involve genetic damage or mutation in critical genes related to the control of cell division, cell death, metastatic potential and activation of signalling or metabolic pathways that give the cells favorable growth and survival characteristics. Many chemical, physical and biological environmental agents are able to interact with DNA to induce mutations.[4]

The most common life style factors that are associated with genetic damage include using tobacco, alcohol consumption, vitamin deficiency, and also metabolic disturbances most commonly like Diabetes Mellitus. Tobacco used in any form can result in irreparable genetic damage and is the most common chemical carcinogen for oral cancer. Diabetes mellitus is a growing and massively silent epidemic that has the potential to cripple health services in all parts of the world. Type-1 insulin dependent and Type-2 insulin resistant type accounts for only 5-10% and 90-95% of all diabetic patients respectively.[5, 6]

Micronuclei (MN) are small nuclei that are originated from chromosome fragments on whole chromosomes enclosed within nuclear membrane during cell division. They may be induced by exposure to carcinogenic agents, oxidative stress and genetic defects in the cell cycle. MN test can be of unquestionable importance and can be used as a cytogenic biomarker to assess the genotoxic effects in exfoliated buccal mucosa cells and is a cost-effective and accurate procedure, which can be easily carried out for population-based studies.[7]

MN can be easily, detected under light microscope using DNA specific (Feulgen, Acridine orange) and non DNA specific (Giemsa, PAP) stains. Giemsa stain was most commonly used stain but Papanicolaou (PAP) stain also used to evaluate MN because of its polychromatic, transparent staining reaction with crisp nuclear and cytoplasmic features.[8] Hence; under the light of above mentioned data, the present study was undertaken for assessing micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco smoking habit.

MATERIALS & METHODS

The present study was conducted in the department of oral pathology of the dental institute and it included evaluation of micronuclei frequency in exfoliated buccal mucosal cells in Type-2 diabetes patients with tobacco smoking habit. Ethical approval was obtained from institutional ethical committee and written consent was obtained from all the patients after explaining in detail the entire research protocol. The 40-50 age group of both genders were included. They were selected from among the patients who reported to Department of oral medicine and radiology of BBDCODS and Type-2 Diabetic patients from Dr. Ram Manohar Lohia Institute and Hospital, Gomti Nagar, Lucknow. After explaining the experimental protocol; their willingness to participate was considered with written /verbal informed consent.

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

- Group 1: 25 Diabetic with tobacco smoking habit
- Group 2: 25 Non-diabetic with tobacco smoking habit
- Group 3: 25 Controls

Relevant history of each patient, including their oral habits, will be recorded thoroughly**.** Age-and sex-matched healthy subjects having no obvious oral lesions or habits of consumption of tobacco, other tobacco- related substances, or potentially toxic substances will be selected as control group. 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. 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. All the results were summarized in Microsoft excel sheet and were analysed by SPSS software.

RESULTS

In the present study, a total of 75 subjects were enrolled. Mean age of the patients of group 1, group 2 and group 3 patients was found to be 45.64 years, 45.88 years and 46.48 years respectively. There were 14 males, 13 males and 14 males in group 1, group 2 and group 2 respectively. The mean MN frequency PER HPF GIEMSA increase with increase in disease severity (normal to type-2 diabetic) and tobacco use. Comparing the mean MN frequency PER HPF GIEMSA of three groups, ANOVA showed significantly different MN frequency PER HPF GIEMSA among the groups.

Table 1: Demographic data

Group	Mean age (years)
Group 1	45.64
Group 2	45.88
Group 3	46.48

Table 2: Gender-wise distribution

Gender	Group 1	Group 2	Group 3
Males	14	13	14
Females	11	12	11

Table 3: MN frequency per high power field GIEMSA

Group	Mean	p- value
Group 1	1.25	0.000 (Significant)
Group 2	1.02	
Group 3	0.72	

Table 4: MN frequency per high power field PAP

Group	Mean	p- value
Group 1	4.26	0.010 (Significant)
Group 2	2.13	
Group 3	0.96	

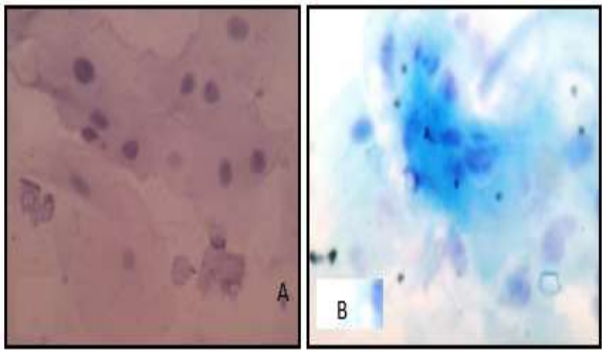

Figure 1: A) Micronuclei in PAP Stain, B) Micronuclei in Giemsa stain

DISCUSSION

The term 'Diabetes Mellitus' in clinical terminology, is a serious and growing health care problem worldwide and is associated with severe acute and chronic complications. Whereas Type-2 Diabetes mellitus is characterized by insulin resistance and relative insulin deficiency is the most prevalent form explaining 90–95% of cases. Type 2 diabetes occurs when the body does not effectively use the insulin produced. The prevalence of Type-2 Diabetes Mellitus is 11% in urban areas while it is 3-9% in rural areas. There are studies which have found prevalence as high as 13.2% in rural areas.[9]

The prevalence of diabetes is higher in men than women. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age. According to Aziza H. Eldarrat (2011) the recent rise in diabetes is not solely a genetic shift but also an environmental shift as a result of lifestyle habits. Its prevalence is increasing epidemically worldwide. By 2030, 366 million people are expected to suffer from diabetes as declared by WHO.[10]

Oral carcinogenesis is a multistep process of accumulated genetic damage leading to cell dysregulation with disruption in cell signalling, DNA-repair, and cell cycle events, which are fundamental to hemostasis. The micronucleus in oral buccal cells is considered to be a biomarker of chromosomal damage caused by genotoxic agents from substances related to tobacco, tobacco itself, and alcohol. The induction of micronucleated cells by carcinogens and mutagens is a sign of the genotoxic effect of such substances. Increased frequency of nuclear aberrations in buccal mucosal cells of tobacco and alcohol users indicates a high risk group of oral cancer.[11, 12]

In the present study genotoxic effects were studied by assessing micronuclei in diabetes as well as tobacco users as genomic damage occurs due to increased ROS and lipid per oxidation in diabetic patients with poor glycemic control and hypertriglyceridemia. Reactive Oxygen Species, including O2-, OH, and H_2O_2 are highly reactive and capable of damaging cellular macromolecules, including proteins, lipids and DNA as explained by Suresh KG Shettigar. According to Tolbert PE, Shy CM, Allen JW(1992) majority of degenerative and developmental diseases are caused by genomic damage, which is produced by environmental exposure of radiation, chemicals, micronutrient deficiency, and lifestyle factors, for example, alcohol, smoking, drugs, gutkha, pan masala and stress. So it is important to bio-monitor, identify, and treat the diseases caused by, or associated with genetic damage.[13, 14]

In the present study, the mean MN frequency PER HPF GIEMSA increase with increase in disease severity (normal to type-2 diabetic) and tobacco use. Comparing the mean MN frequency PER HPF GIEMSA of three groups, ANOVA showed significantly different MN frequency PER HPF GIEMSA among the groups. Exfoliated buccal cells are used to assess genetic damage i.e. Micronuclei (MN) in the present study as oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. MN is induced in oral exfoliated cells by a variety of substances, including genotoxic agents and carcinogenic compounds in tobacco, betel nut, and alcohol. Tobacco-specific nitrosamines have been reported to be potent clastogenic and mutagenic agents which are thought to be responsible for the induction of chromatid/chromosomal aberrations resulting in production of MN.T2 DM is a strong determinant of chromosomal DNA damage in both type-1 diabetes mellitus and T2DM. Buccal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products. Approximately 90% of human cancers originate from epithelial cells. These events can be conveniently studied in the buccal mucosa, which is an easily accessible tissue for sampling in a minimally invasive manner and does not cause undue stress to study subjects.[14- 16]

Microscopically micronuclei are generally assessed using DNA specific stains due to their ability to bind specifically to the DNA and clear identification, but in the present study simple cost effective non specific DNA stains like PAP (Papanicolaou stain) and May Grunewald Giemsa (MGG) is used as these stains have good clarity and transparency of epithelial cells which enable to identify MN easily.[15, 16]

Our findings are in accordance with the findings of Stich, Stich and Parida (1982) observed increased frequency of MN as compared to non chewers and they concluded that MN frequencies in exfoliated buccal mucosal cells seem to represent a useful internal dosimeter for estimating exposure to genotoxins and implication of carcinogenic agents in tissues from which cancers will develop.[17]

Khanna S et al[12] DNA specific stains are preferred for staining nuclei, MN, and other nuclear anomalies in buccal exfoliated cells. Feulgen- Fast Green is favoured by many investigators because of its DNA

specificity and a clear transparent appearance of the cytoplasm which enables easy identification of MN; however method is relatively lengthy and may lead to the underscoring of MN. Other stains include fluorescent dyes, such as diamidino-2-phenylindole (DAPI), acridine orange, Hoechst, and propidium iodide and nonspecific stains like May- Grunewald Giemsa (Giemsa), PAP, haematoxylin and eosin, and Orcien. Some of the studies reported increased frequencies of MN with Giemsa staining and suggest the possibility that cellular structures resembling MN, such as Keratohyaline granules or bacteria, can lead to false positive results. Bacteria can be differentiated from MN by their characteristic shape, smaller size, colour, staining intensity, and their presence upon and between buccal cells on the slide.[12]

Gosavi et al compared the micronuclei index in potentially malignant disorders and malignant cases among tobacco users in cytological smears using papanicolae (PAP) and MGG stains and compared the two techniques. Their results showed an average increase in MN frequencies in potentially malignant disorders as compared to tobacco users without any lesion suggesting that MN is a biomarker of neoplastic progression. Also a better MN frequency was obtained with PAP staining technique. Thus they concluded MN can be used as a noninvasive early detection tool for mass screening, patient education and to check for the efficacy of treatment with PAP stain as the preferred method as compared to MGG.[18]

CONCLUSION

From the above results, the authors concluded that Type-2 DM patients have significantly more genetic damage (in terms of MN frequency). This indicates than MN may be a useful constituent in a panel of biomarkers for the risk of diabetes.

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