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Cytogenetic bio-monitoring study in diabetes patients by Buccal cytome assay in exfoliated buccal epithelial cells

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ABSTRACT

Diabetes mellitus is one of the most common metabolic disorders. It affects millions of people worldwide. Diabetes causes include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. The study was undertaken to compare the frequencies of nuclear abnormalities in buccal cells between diabetic and non-diabetic individuals. 20 diabetic and 20 non-diabetic individuals of both sexes participated in this parallel, randomized, intervention trial. Cytogenetic damage in buccal cells was assessed by using the buccal cytome (Bcyt) assay. It was observed that micronucleus (MN) frequency was significantly higher in participants with diabetes (8.62 ± 0.52) compared with non-diabetic individuals (0.36±0.14). Significant increase was observed in other nuclear abnormalities e.g., binucleated cells (5.60±0.72) and cells with nuclear bud (6.40 ± 0.59) in diabetic individuals in comparison to non-diabetic participants binucleated cells (0.46 ± 0.18), nuclear buds (0.29 ± 0.12). When we compared all the three parameters of Bcyt assay among diabetic and non-diabetic individuals, statistically significant (P<0.001) were observed.

Keywords: Diabetes mellitus, micronuclei, binucleated cell, nuclear bud, buccal cytome assay.

Introduction

Diabetes mellitus is one of the most common chronic endocrine metabolic disorders and a growing health care problem worldwide (Jajarm *et al.*, 2008; Mullner *et al.*, 2013). In India with more than 62 million diabetic individuals currently diagnosed with the disease (Kaveeshwar *et al.*, 2014; Prasad *et al.*, 2010). According to the predictions of Wild et al. Kaveeshwar *et al.*, 2014; Wild et al., 2004, the prevalence of diabetes will double globally from 171 million in 2000 to 366 million in 2030 with a India noticing the highest increase.

Diabetes mellitus is basically characterized by absolute or relative insulin deficiency leading to chronic hyperglycemia, associated with disturbances in the metabolism of carbohydrates, proteins and lipids (Prasad *et al.*, 2010). Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. Accordingly, it may be classified into two broad types; diabetes mellitus type 1, also known as 'insulin dependent diabetes mellitus' (IDDM) is a form of diabetes mellitus resulting from the autoimmune destruction of the insulin-producing beta cells in the pancreas and diabetes mellitus type 2, also known as 'non-insulin-dependent diabetes mellitus' (NIDDM) refers to the form of diabetes mellitus in caused due to insulin resistance and relative lack of insulin. Type 2 diabetes primarily caused due to obesity claims about 90% of cases of diabetes, with the other 10% due primarily to diabetes mellitus type 1 and gestational diabetes.

Besides damaging the kidneys, eyes, nerves, blood vessels, and heart, long standing hyperglycemia can also be associated with buccal alterations such as periodontal disease and many other alterations that can appear before and sometimes predispose to periodontal disease, like impaired function of the salivary glands that lead to a reduction of salivary flow and changes in saliva's composition, taste alterations, burning mouth, greater tendency to buccal infections, delayed healing process, decays, coated tongue and halitosis (Negrato *et al.*, 2010).

The general symptoms of diabetes being so harmless many a times go undiagnosed. Some common

symptoms include frequent urination, excessive thirst and hunger, unusual weight loss, increased fatigue and blurry vision. Currently, diabetes is diagnosed by evaluating the blood glucose levels. The most common diagnostic tests for diabetes being either a random blood sugar estimation or analysis of fasting/post-prandial blood sugar levels. However, monitoring of glycosylated hemoglobin (GHb) levels has become much common recently which gives an accurate and objective measure of glycemic control over the past 3 months without being affected by factors like diet and medications. Hence, GHb estimation is preferred by many to monitor diabetic patients (Prasad *et al.*, 2010).

Hyperglycemia the major abnormality associated with diabetes mellitus can be very often associated with several oral complications. Tissue repair mechanism is damaged and a malfunctioning of the oral mucosa may be due to the alterations in salivary flow (Jajarm *et al.*, 2008; Chavez *et al.*, 2001; Andreassi *et al.*, 2011; Negrato *et al.*, 2010) and its constituents, nutritional changes and weakened immune defenses leading to changes in the microbial oral flora that might result in a greater chance of infections (Jajarm *et al.*, 2008; Little *et al.*, 2002; Southerland *et al.*, 2005). These might be the contributing factor behind the cases of an enhanced number of dental caries, gingivitis and periodontitis, periapical abscess, parotid enlargement, and burning mouth syndrome in diabetics. It has been shown that diabetes may also cause various changes in the cells of the oral mucosa (Thomas *et al.*, 2009), which can be determined by exfoliative cytology which is a straight forward and non-invasive diagnostic method (Prasad *et al.*, 2010; Alberti *et al.*, 2003, Jajarm *et al.*, 2008; Sugerman *et al.*, 1996).

The buccal micronucleus cytome assay is a non-invasive method for studying DNA damage, chromosomal instability, cell death and the regenerative potential of buccal mucosal tissue, and is widely used in bimonitoring studies (Thomas *et al.*, 2009). The major advantage of micronucleus test over other techniques are that it can be applied to interphase cells and doesn't required cell culture or the preparation of metaphase cells. Micronucleus test is cross effective and also time saving.

Micronuclei are small chromatin bodies that appear in the cytoplasm by the condensation of acentric chromosome fragments or by whole chromosome, lagging behind the cell division. Micronucleus assay in buccal epithelial cell has shown to be a sensitive method for monitoring genetic damage in human population (Majer *et al.*, 2001). Exfoliated epithelial cells have traditionally been used for cancer screening and bimonitorig of genotoxic effects in human (Guzman *et al.*, 2003).

In the light of the above discussion, the proposed study was undertaken with the objective to study micronuclei as a potential biomarker to trace genomic damage in diabetic patients and to come up with a diagnostic tool of simplified nature that could help us in detecting diabetes.

METHODS

Study area and sample population:

The study was approved by the Institutional Ethics Committee of Assam University, Silchar. Samples of buccal cells were collected from both patients suffering from diabetes mellitus and normal healthy individuals, the latter serving as control. The study thus considered was in and around Silchar, Assam, India. The present study was performed on twenty patients previously diagnosed to be suffering from diabetes mellitus and twenty non-diabetic healthy individuals with the least risk of developing the disease. A questionnaire was designed that the test individuals had to complete, including age, race, sex and relevant medical history. Individuals who smoked, were alcoholic or had anemia or malignancy were excluded from the study as such conditions may affect cellular shape and morphology.

Test chemicals:

For the present study, we used 0.9% NaCl solution for buccal cell washing, methanol and acetic acid (3:1) for fixing, Leishman stain for staining and distilled water for various dilutions.

Buccal cytome (BCyt) assay:

Buccal cytome assay was performed by following the method of Thomas *et al.*, 2009. Buccal cells were collected from the buccal mucosa using pre-moistened cotton swab by rotating in a circular motion against the cheeks. The cells were then collected in microcentrifuge tubes containing 0.9%

NaCl solution which were then subjected to centrifugation at 2000 rpm for 10 minutes. The supernatant was then discarded carefully without disturbing the cell pellet that by then appears at the bottom of the tube. It was followed by another session of centrifugation at 2000 rpm for 10 minutes and subsequent decantation. The cell solution was then again centrifuged at 1000 rpm for 5 minutes and the supernatant was discarded. The content of the tubes were then agitated using a disposable syringe and smears were prepared using the contents of the tube. Once the slides dried, they were treated with a 3:1 solution of methanol and glacial acetic acid was used as a fixative. The slides were again dried and then stained with 10 drops of Leishman stain for 10 minutes. After a thorough wash in distilled water and subsequent drying, the slides were analysed for cytomorphometric defects under a light compound microscope.

Scoring criteria for buccal cytome assay:

The exfoliated buccal cells were scored as per Thomas et al., 2007. Bcyt assay were determined based on criteria outlined by Tolbert et al., 1991. In each slide, 1000 clearly defined cells with distinct staining were selected manually in a random fashion from different fields, and in order to avoid measuring and counting the same slide twice, the slide is moved in an orderly manner from left to right and then down and across in a stepwise manner on the stage. We scored normal differentiated cells, micronucleated cells, binucleated cells and nuclear buds in our study by using a light-microscope (Leica DMLS) at 1000 magnification.

The different types of cells thus observed under the microscope includes-

Normal differentiated cells: Normal differentiated cells have a uniformly stained nucleus, which is oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nucleus-to-cytoplasm ratio. No other DNA containing structures apart from the nucleus are observed in these cells. These cells are considered to be terminally differentiated relative to basal cells, as no mitotic cells are observed in this population.

Cells with micronuclei: Cells with micronuclei are characterized by the presence of both a main nucleus and one or more smaller nuclear structures called micronuclei (MNi).The micronuclei are round or oval in shape and their diameter should range between 1/3 and 1/16 of the main nucleus. MNi have the same staining intensity and texture as the main nucleus. Most cells with MNi will contain only one MN but it is possible to find cells with two or more MNi. Cells with multiple MNi are rare in healthy subjects but become more common in individuals exposed to radiation or other genotoxic agents. The nuclei in micronucleated cells have the morphology of nuclei in normal cells. The MNi must be located within the cytoplasm of the cells. The presence of MNi is indicative of chromosome loss or fragmentation occurring during earlier nuclear division.

Binucleated cells: Binucleated cells are cells containing two main nuclei instead of one. The nuclei are usually very close and may touch each other and usually have the same morphology as that observed in normal cells. The significance of these cells is unknown, but they are probably indicative of failed cytokinesis.

Cells with nuclear buds: Cells with nuclear buds contain nuclei with an apparent sharp constriction at one end of the nucleus suggestive of a budding process, i.e., elimination of nuclear material by budding. In the original Tolbert et al. these were referred to as 'broken egg' cells. The nuclear bud and the nucleus are usually in very close proximity and appear to be attached to each other. The nuclear bud has the same morphology and staining properties as the nucleus; however, its diameter may range from a half to a quarter of that of the main nucleus. The mechanism leading to nuclear bud formation is not known but it may be related to the elimination of amplified DNA or DNA repair.

Statistical analysis:

All the data were expressed as the mean ± standard error. Student's t test was used to determine the significance of the cellular parameters using Graph pad Prism software. The level of significance was taken as $P < 0.001$.

RESULTS

Analysis of exfoliated buccal cells of control and diabetic patients by buccal cytome assay: Buccal cytome assay (Thomas *et al.*, 2007) was used for

biomarker evaluation to assess the extent of cytological damage in diabetic patients and compared with control individuals. 40 participants irrespective of age, gender and profession were included in the study. Individuals were divided into two groups on the basis of diabetic and non-diabetic patients (Table 1).

Demographic characteristics of the participant population such as name, address, age, family income, religion, occupation, number of family members, body weight, education and details about the chewing habit was recorded as per the questionnaire including various parameters and Body mass index, information about oral health and other information's were recorded.

Table 1: Characteristics of the study groups.

Groups	Diabetic/Non-Diabetic	Male	Female	Total Samples (n)
Group I	Non-Diabetic	10	10	20
Group II	Diabetic	10	10	20

The cytome assay parameters studied are as follows:

Micronucleus (MN):

Table 2 indicates the MN frequency for the studied group. The percentage of MN cells for Group II was 8.62 when compared to Group I 0.36 and found to be statistically significant when compared with control ($P<0.001$).

Nuclear Bud (NBUD):

Table 2 describes the incidence of cells with NBUD among different groups. A statistically significant increase in incidence of cells with NBUD was observed when compared between Group II and Group I ($P<0.001$), indicating genotoxic effects of chewing tobacco.

Binucleated Cells (BN):

The BN cell frequency for each study group is presented in Table 2. Group II (5.60 ± 0.72) show statistically significant higher incidence of BN when compared to control Group I.

Table 2: Incidence of micronucleated cells, binucleated cells and nuclear buds in buccal epithelial cells of two groups.[≠]

Study group	Total Samples (n)	% Micronucleated cells (Mean±SE)	% Binucleated cells (Mean±SE)	% Nuclear buds (Mean±SE)
Group I	20	0.36 ± 0.14	0.46±0.18	0.29±0.12
Group II	20	8.62 ± 0.52*	5.60±0.72*	6.40±0.59*

[≠]Each value indicates Mean ± SE. Statistical analysis: One-way ANOVA.

Values are significantly different from control: *$P < 0.001$.

DISCUSSION

The buccal epithelial cells have been considered to be more sensitive than lymphocytes to any genotoxic agents and they can be collected (Salama and Serrana, 1999). Micronuclei are the potential biomarkers and used widely for biomonitoring study of genotoxic effects in humans (Guzman et al., 2003). The results of this study show that levels of buccal MN in diabetic individuals are approximately 2-fold higher than in non-diabetic participants. MN in exfoliated buccal cells are a novel and non-invasive biomarker of genomic stability, formed during mitosis in the basal cell layer of the epithelium and represent the loss of chromosome fragments or a whole chromosome that failed to be incorporated in the main nuclei (Thomas et al., 2009). Genomic instability is a hallmark of tumourigenesis (Shen, 2011); in addition, cancer patients show increased levels of buccal MN frequency (Burgaz, 2011) compared with healthy individuals. Therefore, our results strongly suggest higher genomic instability in patients with diabetes mellitus compared with non-diabetic participants, which is consistent with epidemiological data indicating increased cancer risk in individuals with type 2 diabetes (Vigneri, 2009). Insulin resistance is among other factors a link between abdominal obesity and hyperglycaemia. High glucose levels may contribute to decreased genomic stability via increased cell proliferation, enhancing the risk of genetic errors and the possibility of cancer development (Natarajan, 1992). Glucose is also used as an energy substrate in tumour cells and might thereby have a direct tumour promoting effect (Polet, 2013).

Biomarkers serve as indicators of environmental or occupational exposures and have the potential for prevention of effects of carcinogen exposure by early detection (Smith et al., 1993). Genotoxic potential of *Sadagura*, a form of smokeless tobacco preparation practiced in the region southern assam was confirmed by the method of buccal cytome assay (Kausar et al., 2009).

In conclusion, the results of this study provide important novel information regarding associations between waist circumference, glucose metabolism and genomic damage. The mentioned associations with MN frequency were observed in a population of diabetic and non-diabetic individuals. Human buccal mucosa is composed of progenitor and maturing cell populations (Ten Cate *et al.*, 1998). Exfoliated cells of buccal mucosa are good indicators of chromosomal damage and other nuclear abnormalities such as binucleates, karyorrhexis and karyolysis (Tolbert *et al.*, 1992).Further studies are required to determine the extent of genetic damage in diabetic patients and the mechanism behind the genetic damage.

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REFERENCES

1. Alberti S., Spadella C. T., Francischone T. R., Assis G. F., Custard T. M., Taveira L. A. (2003). Exfoliative cytology of the oral mucosa in type II diabetic patients: morphology and cytomorphometry. Journal of Oral Pathology and Medicine. 32, 538–543.
2. Andreassi M. G., Barale R., Iozzo P., Picano E. (2011). The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. Mutagenesis. 26(1), 77-83.
3. Burgaz S., Coskun E., Demircigil G. C., Kocabas N. A., Cetindag F., Sunter O., Edinsel, H. (2011). Micronucleus frequencies in lympho-cytes and buccal epithelial cells from patients having head and neck cancer and their first-degree relatives. Mutagenesis. 26, 351–356.
4. Chavez E. M., Borell L. N., Taylor G. W., Ship J. A. (2001). A longitudinal analysis of salivary flow in control subjects and older adults with type 2 diabetes. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology. 91, 166-173.
5. Guzman P, Sotelo-Regil RC, Mohar A, Gonsebatt ME (2003). Positive correlation between the frequency of micronucleated cells and dysplasia in Papanicolaou smears. Environmental and Molecular Mutagenesis. 41, 339-43.
6. Jajarm H. H., Mohtasham N., Rangiani A. (2008). Evaluation of oral mucosa in type II diabetic patients by an exfoliative cytology method. Journal of Oral Science. 50(3), 335-340.
7. Kausar A., Giri S., Mazumdar M., Giri A., Roy P., Dhar P., 2009. Micronucleus and other nuclear abnormalities among betel quid chewers with or without sadagura, a unique smokeless tobacco preparation, in a population from North-East India. Mutat. Res. 677, 72-75.
8. Kaveeshwar S. A., Cornwall J. (2014). The current state of diabetes mellitus in India. Australian Medical Journal. 7(1), 45-48.
9. Little J. W., Falace D. A., Miller C. S., Rhodis N. L. (2002). Dental management of the medically compromised patient. 6th ed., Mosby, St Louis, 320-322.
10. Majer B. J., Laky B., Knasmuller S., Kassie F. (2001). Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage an in chemopreventive trials. Mutation Research. 489: 147–172.
11. Mullner E., Brath H., Nersesyan A., Marlies N., Petschning A., Wallner M., Knasmuller S., Wagner K. (2013). Nuclear anomalies in exfoliated buccal cells in healthy and diabetic individuals and the impact of a dietary intervention. Mutagenesis, 29(1). 1-6.
12. Natarajan R., Gonzales. N., Xu L., Nadler J. L. (1992). Vascular smooth muscle cells exhibit increased growth in response to elevated glucose. Biochemistry Biophysics Research Communications. 187, 552–560.
13. Negrato C. A., Tarzia O. (2010). Buccal alterations in diabetes mellitus. Diabetology and Metabolic Syndrome. 2, 3.
14. Ogden G. R., Cowpe J. G., Wight A. J. (1997). Oral exfoliative cytology: review of methods of assessment. Journal of Oral Pathology and Medicine. 26, 201–205.
15. Prasad H., Ramesh H., Balamurali P.D. (2010). Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. Journal of Cytology. 27(4), 113-117.
16. Polet F., Feron O. (2013). Endothelial cell metabolism and tumour angiogenesis: glucose and glutamine as essential fuels and lactate as the driving force. Journal Internal Medicine. 273, 156–165.
17. Rosin M. P., Gilbert A. M. (1990). Modulation of genotoxic effects in humans. In: Meldelsohn M. L., Albertini R. J., editors. Mutation and Environment, Part E: Environmental Genotoxicity, Risk and Modulation. New York: Wiley-Liss. 351–60.
18. Salama SA, Serrana M, Au WW (1999). Biomonitoring using accessible human cells for exposure and health risk assessment. *Mutat Res*, 436: 99–112.
19. Shen Z. (2011). Genomic instability and cancer: an introduction. Journal of Molecular Cell Biology. 3, 1–3.
20. Smith A. H., Hopenhayn-Rich C., Warner M., Biggs M. L., Moore L., Smith M. T., 1993. Rationale for selecting exfoliated bladder cell micronuclei as potential biomarkers for arsenic genotoxicity. J. Toxicol. Environ. Health 40, 223-234.
21. Southerland J. H., Taylor G. W., Offenbancher S. (2005). Diabetes and Periodontal Infection: Making the Connection. Clinical Diabetes. 23(4), 171-178.
22. Sugerman P. B., Savage N. W. (1996). Exfoliative cytology in clinical and oral pathology. Australian Dental Journal. 41, 71-74.

23. Ten Cate A. R., Ten Cate R., Copelad E. (1998). Oral Histology: Development Structure and Function. Mosby-Year Book, St. Louis, MO.
24. Thomas P., Harvey S., Gruner T., Fenech M. (2007). The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. Mutation Research. 638, 37–47.
25. Thomas P., Holland N., Bolognesi C., Volders M. K., Bonassi S., Zeiger E., Knasmueller S., Fenech M. (2009). Buccal micronucleus cytome assay. Nature Protocols. 4(6), 825-837.
26. Tolbert P. E., Shy C. M., Allen J. W. (1992). Micronuclei and other nuclear abnormalies in buccal smears: methods development. Mutation Research. 271(1), 69–77.
27. Vigneri P., Frasca F., Sciacca L., Pandini G., Vigneri R. (2009). Diabetes and cancer. Endocrine Related Cancer. 16, 1103–1123.
28. Wild S., Roglic G., Green A., Sicree R., King H. (2004). Global prevalence of diabetes-estimates for the year 2000 and projections for 2030. Diabetes Care. 27(3), 1047-1053.

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