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Nuclear abnormalities in buccal mucosa cells of patients with type I and II diabetes treated with folic acid

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ABSTRACT

Diabetes mellitus (DM) is characterized by high blood glucose. Excessive production of free radicals may cause oxidative damage to DNA and other molecules, leading to complications of the disease. It may be possible to delay or reduce such damage by administration of antioxidants such as folic acid (FA). The objective of this study was to determine the effect of FA on nuclear abnormalities (NAs) in the oral mucosa of patients with DM. NAs (micronucleated cells, binucleated cells, pyknotic nuclei, karyorrhexis, karyolysis, abnormally condensed chromatin, and nuclear buds) were analyzed in 2000 cells from 45 healthy individuals (control group) and 55 patients with controlled or uncontrolled type I or II DM; 35 patients in the latter group were treated with FA. Samples were taken from the FA group before and after treatment. An increased rate of NAs was found in patients with DM in comparison with that of the control group ($P < 0.001$). FA supplementation in patients with DM reduced the frequency of NAs (20.4 ± 8.0 before treatment vs. 10.5 ± 5.2 after treatment; $P < 0.001$). The type I and type II DM and controlled and uncontrolled DM subgroups were analyzed in terms of sex, age, and smoking habit. The significantly reduced frequencies of buccal mucosa cells with micronuclei, binucleation, pyknosis, karyorrhexis, karyorrhexis + abnormally condensed chromatin, karyolysis, and nuclear buds produced by FA supplementation in DM patients ($P < 0.02$) are consistent with the idea that free radicals are responsible for the increased frequency of NAs in DM patients.

1. Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia resulting from defects in insulin secretion and/or insulin action. DM is a progressive condition associated with complications such as atherosclerosis, kidney disease, nerve damage, and blindness [1–4]. The World Health Organization has predicted that there will be approximately 360 million diabetics in the world by 2030. Furthermore, according to data from the Ministry of Health, 9% of the adult population in Mexico is diabetic [5–7].

Chronic hyperglycemia increases production of superoxide radicals, which contribute to the complications of diabetes [3]. Increased production of free radicals and reactive oxygen species (ROS) causes oxidative damage to biomolecules [1,3,8–11]. ROS can damage genetic material, resulting in abnormalities in the integrity of the cell nucleus and DNA breakage, producing micronuclei (MN) [8–10,12–16]. Antioxidants can counteract DNA damage generated by free radicals in patients with diabetes and other conditions [3,4,8,10,17–19].

Folic acid (FA) is an important nutrient in relation to cardiovascular disease and birth defects [20,21]. FA is involved in amino acid

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Table 1
General characteristics of the participants.

General characteristics	Total		Healthy controls		Diabetic patients					
							Type I DM patients		Type II DM patients	
	n	Age (years)	*n* (%)	Age (years)	*n* (%)	Age (years)	*n* (%)	Age (years)	*n* (%)	Age (years)
Total	100	36.30 ± 14.82	45 (45.0)	32.84 ± 12.08	55 (55.0)	39.13 ± 16.29	32 (58.2)	30.00 ± 12.64	23 (41.8)	51.83 ± 11.69
Women	61	36.43 ± 15.46	28 (62.2)	32.46 ± 11.47	33 (60.0)	39.79 ± 17.65	19 (59.4)	27.42 ± 10.10	14 (60.9)	56.57 ± 9.96
Men	39	36.10 ± 13.95	17 (37.8)	33.47 ± 13.37	22 (40.0)	38.14 ± 14.35	13 (40.6)	33.77 ± 15.29	9 (39.1)	44.44 ± 10.67
Smokers	34	34.94 ± 13.02	16 (35.6)	34.63 ± 10.92	18 (32.7)	35.22 ± 14.95	11 (34.4)	25.73 ± 6.68	7 (30.4)	50.14 ± 11.59
Non-smokers	66	37.00 ± 15.71	29 (64.4)	31.86 ± 12.76	37 (67.3)	41.03 ± 16.77	21 (65.6)	32.24 ± 14.49	16 (69.7)	52.56 ± 12.03
Controlled disease					16 (29.1)	37.31 ± 17.20	10 (31.2)	28.50 ± 13.62	6 (26.1)	52.00 ± 11.83
Uncontrolled disease					39 (70.9)	39.87 ± 16.07	22 (68.8)	30.68 ± 12.44	17 (73.9)	51.76 ± 12.01

Data of age is expressed as mean ± standard deviation. *n*: sample size. Controlled disease (HbA1 < 7%). Uncontrolled disease (HbA1c > 7%).

metabolism, nucleic acid biosynthesis, and methylation reaction; it is necessary for maintaining cellular homeostasis and FA deficiency can cause disease.

Some nuclear abnormalities (NAs) in oral mucosa cells serve as biomarkers of accelerated aging, cancer, and neurodegenerative diseases [22–24]. The buccal micronucleus cytome assay is an alternative test used to evaluate genotoxicity in epithelial cells [23,24–28], where the most suggestive alterations in the morphology of neoplastic cells are produced in the nucleus. The size, density, and distribution of chromatin undergo modifications, which lead to genome damage. MN, indicating a loss of genetic material, is a buccal mucosa NA. Nuclear extensions and nuclear buds (NBUDs), which can generate MN, have a source similar to MN and are associated with the gene amplification process [23,26,29–31]. Binucleated cells (BN) are caused by defects in cytokinesis. BN, MN, and NBUDs have been associated with genotoxicity. Pyknosis (PYK, small nucleus), karyolysis (KL, nucleus loss), karyorrhexis (KR, nucleus fragmentation), and abnormally condensed chromatin (CC, lumped nucleus) [20,23,26] are related to cytotoxicity events. PYK and CC are associated with differentiation and maturation of the epithelium; however, an increase in the frequency of NAs is observed in response to cellular damage. PYK, CC, and KR, accompanied by keratinization, occur as an adaptive response to cell damage, e.g. during normal keratinization in the cells of the oral mucosa, which accompanies apoptosis [23], whereas KL has been linked to cell necrosis, which occurs after injury by agents that seriously disturb cell development [23,26].

The present study evaluated whether the administration of FA to patients with diabetes can reduce the frequency of NAs in oral mucosa cells.

2. Materials and methods

2.1. Participants

The study population consisted of 100 individuals divided into two groups. The first group of 55 volunteers were adult patients of any age and either sex, with controlled (glycosylated hemoglobin: HbA1c < 7%) or uncontrolled (HbA1c > 7%) type I or type II DM, as well as a clinical diagnosis and laboratory data from the Endocrinology Service of the Centro Médico Nacional de Occidente (Instituto Mexicano del Seguro Social). Patients who had not taken FA or any other commercial antioxidant, such as vitamin C, vitamin E, vitamin A, lutein, or melatonin, in the past two months were sampled to evaluate the effect of diabetes on NA frequency. From this group, 35 patients with DM received FA supplementation (Lab. Valdecasas, S.A., one tablet of 5 mg FA, three times daily for 30 days), after which a second sample was obtained at the end of FA treatment. Additionally, 45 healthy individuals (with fasting glucose <110 mg/dL) with an age distribution similar to that of the diabetic patients, were chosen as a control group for reference. Individuals with lupus, rheumatoid arthritis, or cancer, or who had been receiving drugs with a micronucleogenic effect, were not included.

2.2. Ethical considerations

This study was performed in accordance with institutional and governmental regulations and was approved by the Local Committee on Health Research (registry number 2002249018). All procedures were performed in accordance with the institutional guidelines of the Centro de Investigación Biomédica de Occidente (Instituto Mexicano del Seguro Social), Guadalajara Jalisco, México, which comply with guidelines approved by national and international institutes of health and the Declaration of Helsinki [32].

All subjects who agreed to participate in the study signed a letter of informed consent. Samples were coded to maintain the confidentiality of the personal information of each individual. During an interview, a detailed questionnaire was used to collect personal information including age, gender, alimentary and smoking habits, intake status of drugs and antioxidants, and medical history.

2.3. Sample preparation and analysis

Samples were collected by scraping the buccal mucosa and spread onto precoded slides in duplicate. The smears were air-dried, fixed in 80% methanol for 48 h, and stained with acridine orange [8,23].

An analysis of cells with MN, BN, PYK, KR, CC, KL, and NBUD was performed in 2000 cells/sample. The cells were scored using an Olympus BX51 fluorescence microscope with a 100 × immersion objective according to established guidelines [23,26]. Because KR and CC are difficult to differentiate when acridine orange staining is used [27], we scored both parameters as a single abnormality (KR + CC) reflecting fragmented nuclei.

For a cell to be considered normal, it had to present an intact and relatively homogeneous cytoplasm, little or no junction with adjacent cells, and an intact and homogeneous nucleus with a smooth and distinct nuclear perimeter. NAs were defined by previously described parameters [23,26] and were evaluated by assessing staining intensity, texture, and the focal plane of the nucleus.

2.4. Statistical analysis

Results are presented as the number of cells with each NA in 1000 cells (‰). Results are presented as the mean and standard deviation of the study group and/or a category of analysis. Data were analyzed with SPSS v. 11.0 (IBM Co., Armonk, NY, USA), which was used to compare sample values for each NA utilizing descriptive analysis, as well as to evaluate the frequency of each NA by means of non-parametric tests. The Mann–Whitney *U*-test was used for independent samples, whereas the Wilcoxon test was used for related samples. The analyses were performed to compare values obtained before and after FA treatment to reference values of healthy subjects, as well as to assess the relationships between NAs and factors such as age, smoking, gender, and diabetes type and control. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. General characteristics of the participants

The general characteristics of all participants with regard to sex, presence or absence of diabetes, and smoking habits are presented in Table 1. The average age of the participants was 36.3 ± 14.8 years. Men accounted for 39% of the study group. The average numbers of NAs were 16.8 ± 9.0 in men and 15.2 ± 10.5 in women ($P > 0.05$).

Oral mucosa samples from 100 participants (smokers and non-smokers), including 45 healthy individuals and 55 patients with diabetes, were analyzed, yielding an average of 16.2 ± 10.1 NAs among the entire study population. In the control group consisting of healthy individuals, the average number of NAs was 10.2 ± 7.7, whereas the average number of NAs in patients with diabetes was 20.4 ± 9.2 (Table 2).