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Frequency of Micronuclei in Mexicans with Type 2 Diabetes Mellitus

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Abbreviations: DM2 – Type 2 diabetes mellitus; MN – Micronuclei; Cyt-B – Cytochalasin-B; HbA_{1c} – glycosylated hemoglobin.

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Abstract: A case–control study was carried out on a sample of 15 Mexican patients (40–56 years old) with type 2 diabetes mellitus (DM2) that had developed five years and been treated with oral hypoglycemic drugs (sulfonylurea and/or metformin), with no microvascular or macrovascular complications. The aim of this study was to assess whether Mexican patients with DM2 differed from a control group in the frequency of micronuclei (MN). A control group of 10 individuals without DM2 (38–54 years old) was included. The frequency of MN in binucleated lymphocytes was analyzed according to the Fenech criteria. At time being this investigation should be considered as a preliminary study in which the influence of potential confounders cannot be adequately assessed. However, our result showed a MN frequency significant increase in DM2 patients (6.53 ± 2.03 per 1000 cells) relative to that of the control group (3.10 ± 1.79 per 1000 cells). MN may constitute a possible component of a panel of biomarkers for the risk of DM2. This cytogenetic damage also indicates an enhanced risk of cancer, as has been found in previous studies. These results should be validated by other researchers.

Introduction

The high prevalence of type 2 diabetes mellitus (DM2) in Mexico represents a public health problem because it is the third highest cause of mortality [1]. DM2 patients display several complications such as the overweight, atherosclerosis, retinopathy, and nephropathy.

DM2 has been associated with elevated levels of DNA damage, increased susceptibility to mutagens, and a decreased efficacy of DNA repair [2], causing genomic instability and consequently cancer [3–5]. However, the mechanism underlying this association is unclear.

The number of micronuclei (MN) has been mainly used as a biomarker in peripheral blood lymphocytes to evaluate genotoxic risks in the work environment. MNs are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosomal fragments or intact whole chromosomes that lag behind at the anaphase stage of cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis [6], and has recently been suggested as a biomarker for cancer risk [7]. The conceptual basis for this application is the idea that increased cytogenetic damage reflects an enhanced cancer risk [8].

In order to assess whether the frequency of MN differs in Mexican patients with and without DM2 a case–control study was carried out.

Material and Methods

Study Population

Fifteen patients (40–65 years old) with a diagnosis of DM2 according to the Latin-American Association of Diabetes [9] and confirmed by impaired fasting

glucose test (>126 mg/dl) and oral glucose test tolerance (>200 mg/dl), which had developed five years, were sampled from the Medical Unit No. 26 of the Instituto Mexicano del Seguro Social (IMSS) in Monterrey, Mexico, during 2004.

Patients with acute myocardial infraction or ischemic coronary disease in the personal historical (revealed by cardiology evaluation) were excluded from the present study. Exclusion criteria included also severe diseases such rheumatic diseases, chronic liver diseases and cancer, and subjects who underwent recent radiological procedures (<one month).

Ten "healthy" individuals (38–54 years old, evaluated with clinical history), without DM2 in the personal history, with fasting glucose test (<100 mg/dl), and oral glucose test tolerance (60 to 100 mg/dl) from the same Medical Unit, were included into the control group.

The 25 unrelated individuals answered a questionnaire related to their lifestyle. The 15 patients with DM2 were treated during five years with oral hypoglycemic drugs (sulfonylurea and/or metformin). Neither the patients nor controls were taking vitamin or antioxidant supplements.

This study was approved by the Ethical Committee of the Centro de Investigación Biomédica del Noreste, IMSS, and each subject gave his/her written informed consent.

Lymphocyte cultures

Peripheral blood samples were collected from patients and controls in sterile heparinized vacutainers. Lymphocytes were cultured for 44 h in McCoy 5A medium supplemented with 2% phytohemagglutinin, 15% fetal calf serum, and antibiotics (streptomycin–penicillin). Cytochalasin-B (Cyt-B) was added to a final concentration of 6.0 ug/mL. Twenty-eight hours after the addition of Cyt-B, the cells were harvested by cytocentrifugation and lymphocytes with intact cytoplasm were collected using a conventional method [10].

MN Test

The cultured lymphocytes were stained with 0.28% Giemsa stain. The slides were then washed in distilled water, cleaned in xylene, and mounted in resin [11].

MN counts

For each subject, 2000 binucleated peripheral lymphocytes were studied by blind analysis under a light microscope with a 100× oil immersion lens. The Fenech criteria for identifying the MN were: (a) a diameter between 1/16 and 1/3 of the mean diameter of the main nucleus; (b) non-refractivity; (c) no linkage or connection to or overlap with the main nucleus; (d) the same staining intensity as the nucleus; and (d) location within the cytoplasm [11].

Statistical Analysis

The statistical comparison of the number of cells with of MN in individuals with and without DM2 was made using the Student's t-test. Sample size for two-sample comparison of means was estimated using MINITAB software (version 15.0) ($\alpha = 0.05$, $1 - \beta = 0.80$, mean difference = 3.43, and Sigma = 2.56). A value of $P < 0.05$ was considered significant.

Results

Table 1 shows a description of the general characteristics of the 15 patients with DM2 and the 10 control individuals. Most were married women (housewives) from the state of Nuevo León, with elementary education, without habit smoking, alcohol or coffee consumption, or the practice of exercise.

The clinical characteristics of the 15 patients with DM2 and the 10 healthy individuals are given in Table 2. The average ages for the patients and controls (average ± standard deviation) were 49 ± 6.7 years (range, 40–56 years) and

Table 1 – Characteristic of the individuals with and without DM2

Characteristics	Diabetes (%) (n=15)	Controls (%) (n=10)
Gender		
Men	5 (33)	2 (20)
Women	10 (67)	8 (80)
Marital Status		
Married	11 (73)	7 (70)
Single	4 (27)	3 (30)
Birthplace		
Nuevo Leon	9 (60)	8 (80)
Outside Nuevo Leon	4 (40)	2 (20)
Education		
None	2 (13)	0
Elementary	10 (67)	6 (60)
Bachelor	3 (20)	4 (40)
Occupation		
Housewives	11 (73)	4 (40)
Blue-Collar	3 (20)	2 (20)
White-Collar	1 (7)	4 (40)
Habit		
Exercise	3 (20) yes, 12 (80) not	1 (10) yes, 9 (90) not
Smoking	4 (27) yes, 11 (73) not	3 (30) yes, 7 (70) not
Alcohol (>2 units/day)	5 (33) yes, 10 (67) not	4 (40) yes, 6 (60) not
Coffee (>4 cups/day)	6 (40) yes, 9 (60) not	5 (50) yes, 5 (50) not
Drugs		
Hypoglycaemic	15 (100) yes	10 (100) not
Antioxidant	15 (100) not	10 (100) not

46 ± 3.8 years (range, 38–54 years), respectively. Thus, the age distributions of the patients and controls were similar. In both groups, the average values for creatinine, cholesterol, albumin, and globulin were similar, but those for HbA_{1c}, glucose, and triglycerides were significantly higher in the patients with DM2 ($P < 0.001$), indicating poor glycemic control.

The numbers of cells with MN were higher in patients with DM2 (6.53 ± 2.03 per 1000 cells) than in control individuals (3.10 ± 1.79 per 1000 cells), as shown in Table 3.

Discussion

The mean number of cells with MN per 1000 cells in the control group was 3.1, which is consistent with the results of a previous study [7], which reported a value of 3.0 in healthy women. Patients with DM2 had a higher frequency of 6.5 cells with MN/1000 cells, relative to the frequency in healthy individuals (3.1), which is according to a previous study [12]. Preliminary results in our laboratory show elevated levels of DNA damage (expressed as % comet tail DNA) in these patients (4.37 ± 0.53) compared to controls (2.41± 0.26). These finding support an increase in the frequency of cells with damaged chromosomes in diabetic patients

Table 2 – Clinical characteristics of individuals with DM2 and healthy individuals

	Diabetes (X±SD)	Controls (X±SD)
Age (years)	49±6.7	46±3.8
BMI (kg/m2)	27.5±1.7	25.3±1.2
Glucose (mg/dl)	209.72±78.11*	98.16±7.12
HbA_{1c} (%)	9.44±1.88*	5.48±0.03
Cholesterol total (mg/dl)	224.79±54.95	221.12±34.63
Creatinine (mg/dl)	0.96±0.46	0.86±0.16
HDL cholesterol (mg/dl)	38.09±12.69	41.82±14.75
LDL cholesterol (mg/dl)	131.88±47.29	143.32±39.97
Triglycerides (mg/dl)	313.43±300.45*	179.82±112.42
Proteins total (mg/dl)	7.46±0.53	7.15±0.38
Albumin (g/dl)	3.98±0.42	4.22±0.30
Globulin (g/dl)	3.51±0.49	2.98±0.47

*P <0.001 when compared to control.

Table 3 – Average number of MN/1000 cells in individuals with and without DM2

Group	n	No. cells studied	MN Average ± SD	MN Range
DM2	15	30,000	6.53±2.03*	4–10
Control	10	20,000	3.10±1.79	1–6

n = number of individuals studied, *t = 4.33, df = 23, P <0.0001 when compared to control.

with poor glycemic control. This may help to explain the strong correlation between high serum glucose concentrations and DNA damage [13].

Several studies, using other techniques, have reported DNA damage in patients with DM2. Using sister chromatid exchange (SCE), Kulkarni et al. [14], reported chromosomal damage in patients with DM2 treated with chlorpropamide, they was statistically significant increase in the individual numbers of SCEs per metaphase in each of the patients treated with the drug compared with the mean of the pooled control values. Sardas et al. (2001) [15] reported significant differences between control and DM2 patients in terms of frequencies of damaged cells, the extend of DNA migration in alkaline comet assay was greater in DM2 patients by comparison with control, which might indicate that DM2 patients are handling more oxidative damage on a regular basis. Blasiak et al. (2004) [16] reported basal endogenous DNA damage evaluated with the alkaline comet assay (measured as the mean tail DNA) in DM2 patients respect to healthy controls, suggesting that DM2 may be associated with the elevated level of oxidative DNA damage.

Conversely, Anderson et al. [17] and Hannon-Fletcher et al. [18] reported no DNA damage in insulin-dependent or non-insulin-dependent patients using the comet assay. This discrepancy may be attributable to the good glycemic control in the latter subjects.

The high frequency of MN observed in patients with DM2 could be due to: (1) an elevated level of oxidative stress, leading to a decrease in glutation synthesis, because the accumulated products of oxidative stress, especially reactive oxygen species, can cause DNA damage [16] ; (2) the downregulation of insulin, an increase in levels of the DNA repair enzyme XPD, or an increase in the extent of DNA damage [19]; and/or (3) some anti-diabetes drugs, such as metformin, which may decrease vitamin B12 levels and increase levels of homocysteine, perhaps by inhibiting its absorption, thus increasing the frequency of MN [20]. *In vivo* and *in vitro* studies with human cells have clearly shown that folate deficiency, vitamin B12 deficiency, and elevated plasma homocysteine are associated with the expression of excessive uracil in DNA, with DNA hypomethylation, and with MN formation [21]. It is necessary to evaluate these factors in further studies to clarify their association with DNA damage in patients with DM2.

The increase in the frequency of cells with MN in patients with DM2 can contribute to genomic instability, and consequently to cancer [22]. In other words, patients with DM2 may have, on average, an increased risk of cancer. Therefore, it is important to estimate this risk, especially in patients with DM2. However, at present, it is not possible to do so.

Epidemiological data suggest a relationship between DM2 and cancer risk, but the mechanism underlying this association is unclear. Perturbation of glucose and insulin regulation may also influence colorectal carcinogenesis, because higher levels of glycosylated hemoglobin, body mass indices, and the use of medications

to treat diabetes correlate with colorectal cancer [23, 24]. Also, recent observational studies have provided consistent evidence on associations of diabetes with increased risk of cancers of the pancreas [25], liver [26], and endometrium [27]. The possible link between diabetes and cancer should also be considered in terms of the contribution made by the insulin and insulin-like growth factor axes, which are major determinants of cellular proliferation and apoptosis [28].

Conclusion

At time being this investigation should be considered as a preliminary study in which the influence of potential confounders cannot be adequately assessed. Interaction between these factors should be carefully conducted in further studies that comprise a sufficient number of subjects. However, our result clearly demonstrated that DM2 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. This cytogenetic damage reflects an enhanced cancer risk, as has been shown in previous studies. Nonetheless, these results should be validated by other researchers.

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