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Elevated frequencies of micronuclei in pregnant women with type 1 diabetes mellitus and in their newborns

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ABSTRACT

Pregestational diabetes mellitus (type 1 and type 2) affects about 1% of the obstetric population. In diabetes, persistent hyperglycemia can be a source of DNA damage via overproduction of reactive oxygen species (ROS). Using the cytokinesis-block micronucleus (CBMN) test, we measured the frequencies of micronuclei (MN) per 1000 binucleated (BN) cells in pregnant women (mothers) with type 1 diabetes mellitus (T1DM) and in their newborns. Peripheral blood lymphocytes were collected from 17 pregnant women with T1DM and cord-blood lymphocytes from their 17 newborns. The control group included 40 pregnant women (mothers) without diabetes mellitus (DM) and their 40 newborns. In the group of pregnant women with T1DM, the mean number of MN per 1000 BN cells was 2.35 (±1.07), significantly ($p < 0.001$) higher than in the control group of pregnant women (0.86 ± 0.90). The frequency value in the group of newborns of T1DM mothers was 1.42 (±0.60), significantly ($p < 0.05$) higher than in the corresponding control group (0.67 ± 0.79). The value in the group of mothers with T1DM was significantly ($p < 0.05$) higher than in their newborns. Comparing mothers without DM with their newborns, no significant frequency differences were observed. No significant correlations were observed between MN frequencies in mothers with T1DM and either the frequencies in their newborns, the duration of diabetes, or HbA_{1C} levels. Our results indicate that T1DM is accompanied by increased frequencies of MN in pregnant women and their newborns.

1. Introduction

Type 1 diabetes mellitus (T1DM) results from cellular-mediated autoimmune destruction of pancreatic β-cells, usually resulting in absolute insulin deficiency [1,2]. T1DM accounts for approximately 5–10% of patients diagnosed with diabetes in the general population [2,3]. Incidences of T1DM are generally higher among populations of European descent than among populations of African or Asian descent [4]. The major forms of diabetes [(T1DM), type 2 (T2DM), and gestational (GDM)] occur in 1–14% of pregnancies in the USA [5,6]. Approximately 7.5% of pregnant diabetic patients have T1DM [7]. Diabetes is the second most common maternal/fetal complication of pregnancy [3,5,7–11].

The role of oxidative stress in the onset, progression, and complications of T1DM and T2DM has been described [12–16]. There are multiple sources of oxidative stress in DM, including nonenzymatic, enzymatic, and mitochondrial pathways [17,18]. Glucose autoxidation, non-enzymatic protein glycation, and the formation of advanced glycation end products (AGEs) have been demonstrated in DM patients [14,19,20]. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate reactive hydroxyl radicals (•OH) [14,17,21]. Overproduction of ROS induces oxidative damage to membrane lipid, proteins, and DNA, including purine and pyrimidine base damages, single-strand breaks (SSBs), double-strand breaks (DSBs), and DNA–DNA

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or DNA–protein cross-links [22–25]. The formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo or 8-OHdG) is the most frequent effect of DNA oxidative damage [26].

Genotoxic effects of ROS overproduction in patients with DM have been detected by measuring urinary 8-OHdG [27–33] and with the comet assay [34–39]. Urinary 8-OHdG is a biomarker of oxidative DNA damage in T1DM [28,30,31] and T2DM [27,29,32], with higher levels in DM patients than in controls. High glycolysated hemoglobin (HbA_{1C}) levels in T1DM patients were associated with high urinary 8-OHdG levels [28,30,31]. Qiu et al. [33] noted that urinary 8-OHdG levels in pregnant women with GDM were significantly higher than in control individuals. Oxidative DNA damage in lymphocytes has been measured with the comet assay in T1DM [34–36] and T2DM patients [35,37–39]. These authors agreed that DNA damage as observed with the comet assay was higher in both types of DM, in comparison to the controls.

Micronucleus (MN) frequency is a sensitive biomarker of genotoxicity [40–42]. To date, few studies have been published estimating MN frequency in patients with T1DM [43,44]. Zuniga-Gonzalez et al. [43] investigated MN frequency in 55 patients with T1DM, including 18 patients with controlled diabetes (HbA_{1C} level <7%) and 37 patients with uncontrolled diabetes (>7%). The authors observed significant increases of MN frequency in both groups, compared to the controls, and higher levels were seen in the patients with uncontrolled disease [43]. Cinkilic et al. [44] did not find a significant increase in MN frequency in the peripheral blood lymphocytes of 35 adult patients with T1DM compared to a control group. In multicenter population-based studies of MN frequencies in 223 healthy pregnant women and their 251 newborns (including multiple pregnancies), Loock et al. [45] noted a significant increase of MN frequency in mothers compared to the newborns. Many different factors affect MN frequency, including genetic predispositions, exposures, and nutrient deficiency or excess [46]. Both population and ethnic differences are observed [45,47]. To date, there have been no studies of MN frequencies in pregnant women with T1DM and in their newborns.

The aim of this study was to evaluate MN frequency in pregnant women with T1DM and in their newborns, exposed in utero to increased oxidative stress due to mother's diabetes. The cytokinesis-block micronucleus (CBMN) test was used as an indicator of chromosome damage in peripheral blood lymphocytes of mothers and in cord blood lymphocytes of their newborns.

2. Materials and methods

2.1. Patients

The approval of the Bioethics Committee of the Medical University of Lodz (RNN/653/10/KB, Nov. 16, 2010) was obtained.

The study group included 17 pregnant women (mothers) with T1DM and their 17 newborns. The control group consisted of 40 pregnant women (mothers) without DM and their 40 newborns. The control-positive group comprised 10 pregnant women without DM and their 10 newborns. Pregnant women in the 1st trimester of pregnancy were administered a daily dose of 400 μg of folic acid for prevention of neural tube defects. Peripheral blood lymphocytes were obtained from mothers and from cord blood lymphocytes of the newborns. In the control-positive group, a known mutagen, chlormethine hydrochloride (Nitrogranulogen) (Sigma, St. Louis, MO, USA) (CAS 55-86-7) was added to the samples in vitro at a concentration of 0.25 μg/mL. The cytokinesis-block micronucleus (CBMN) test was applied in vitro [42,48].

The deliveries took place at the Fetal-Maternal and Gynecology Department, Polish Mother's Memorial Hospital Research Institute in Lodz, Poland. Blood samples were collected from Nov. 2010 to June 2012 and additionally from controls between Dec. 2012 and June 2013. Clinical characteristics of the groups are presented in Table 1.

2.2. Lymphocyte macrocultures and microscopic evaluation

Peripheral blood samples (10 mL) were collected from mothers from the elbow vein and umbilical-cord blood samples (10 mL) from their newborns, under sterile conditions, immediately after separation of the umbilical cord and placenta. For each mother and newborn, two blood cultures were set up and then harvested using standard procedures [49]. Lymphocyte cultures were prepared from heparinized peripheral and cord blood according to the method of Moorehead et al. [50]. For each assay, peripheral or cord blood (10 mL) was allowed to sediment for 2–3 h. The culture medium was Eagle's 1959 (MEM) (Biomed, Lublin, Poland) plus 10% (v/v) fetal calf serum (Biomed, Lublin, Poland) and antibiotics: crystalline penicillin (100 IU/mL) and streptomycin (100 μg/mL) (Gibco, Grand Island, NY, USA) for 72 h at 37 °C. Phytohaemagglutinin M solution (concentration of 1%, v/v) (Gibco, Grand Island, NY, USA) was added to the culture medium (0.1 mL/10 mL) to stimulate the cell division.

Table 1
Clinical characteristics of the investigated groups.

Clinical data	Mothers with T1DM[a] (n = 17)	Mothers without DM[b] (n = 40)
Age at delivery (years)	30 ± 4.2	31 ± 5.5
Height (cm)	165 ± 6.3	167 ± 5.3
Weight (kg)	65 ± 11.71	65 ± 10.05
BMI (kg/m^2) before pregnancy	23.7 ± 3.3	23.4 ± 3.4
Gestational weight gain (kg)	17.2 ± 5.3	14.9 ± 5.1
Diabetic diagnosis age (years)	16 ± 7.6	–
Smoking, n (%)	0 (0%)	5 (12.5%)
Fasting glucose (mg/dL)	103.06 ± 16.27	82.2 ± 8.4
Mean HbA_{1C}[c] (%) 1st trimester	6.3 ± 1.1	–
Mean HbA_{1C} (%) 2nd trimester	6.0 ± 0.9	–
Mean HbA_{1C} (%) 3rd trimester	6.2 ± 0.6	–

Clinical data	Newborns of mothers with T1DM	Newborns of mothers without DM
Gender (n): female/male	7/10	21/19
Gestational age (week)	36.6 ± 3	37.9 ± 3
Weight (g)	3342 ± 865	3100 ± 675
Length (cm)	51.3 ± 10	52.7 ± 5.2
Fasting glucose (mg/dL)	62.0 ± 15	68.2 ± 16
Apgar score in 1st minute	8 ± 1.1	9 ± 1.1
Apgar score in 5th minute	9 ± 0.8	9 ± 0.9
Age of fathers	32 ± 5	33 ± 7

[a] T1DM: type 1 diabetes mellitus.
[b] DM: diabetes mellitus.
[c] HbA_{1C}: glycated hemoglobin is a form of hemoglobin that is formed in a non-enzymatic glycation pathway by hemoglobin exposure to plasma glucose.

2.2.1. Cytokinesis-block micronucleus (CBMN) assay

The CBMN assay was performed following the addition of cytochalasin B (Sigma, St. Louis, MO, USA) at a final concentration of 6 μg/mL, after 44 h of the 72 h incubation period, to prevent cytokinesis. A hypotonic shock was performed at 20 °C for 5 min using 0.075 M KCl (Serva, Heidelberg, Germany). The cells were centrifuged and Carnoy's fixative (methanol: acetic acid, 3:1, v/v) solution was freshly added and dropped on slides. Slides were stained with 2% Giemsa (pH 6.8) (Sigma, St. Louis, MO, USA). Chlormethine hydrochloride (Nitrogranulogen) (Sigma, St. Louis, MO, USA) (CAS 55-86-7), 0.25 μg/mL, was used as positive control. Slides were coded for data entry and observed according to the standard procedure. Only cells with well-preserved cytoplasm were scored. The number of MN per 1000 binucleated (BN) cells was scored for each sample and the % frequency was calculated. We used the criteria for selection of binucleated cells and identification of MN as reported in the HUMN project [42,48].

2.3. Statistical analysis

Arithmetical means and standard deviations (SD) were calculated. The results were subjected to statistical analysis with STATISTICA software. The Shapiro–Wilk test was used to determine the distribution. U Mann–Whitney or Student's t-tests were used when two groups were compared, dependent on the type of distribution. The correlations of the values investigated in the study were estimated with Spearman's rank correlation test and the correlation significance was investigated by the significance test. The level of significance was determined (p). A p-value of less than 0.05 was considered as statistically significant.

3. Results

3.1. Analysis of micronuclei (MN)

The mean numbers of MN per 1000 binucleated cells were 2.35 (±1.07) for T1DM mothers, 1.42 (±0.60) for their newborns, 0.86