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What is the impact of gestational diabetes mellitus on frequency of structural chromosome aberrations in pregnant women and their offspring?

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ABSTRACT

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance which results in hyperglycemia first diagnosed during pregnancy. It is associated with an increased levels of oxidative stress due to overproduction of reactive oxygen species (ROS). Overproduction of ROS induces protein oxidation, lipid peroxidation and different types of DNA damage. The objective of this study was to determine the frequencies of structural chromosome aberrations (CA) in peripheral blood of pregnant women (mothers) with GDM and in cord blood of their newborns. Peripheral blood lymphocytes were collected from 35 GDM mothers and cord blood lymphocytes from their 35 newborns. The control group included 30 pregnant mothers without diabetes mellitus (DM) and their 30 newborns. CA were evaluated with *in vitro* chromosome aberration assays. We observed a moderate increase of the mean numbers of structural CA between GDM mothers and their newborns, GDM mothers and mothers without DM, GDM mothers' offspring and the offspring of mothers without DM, mothers without DM and their newborns, but this effect did not reach statistical significance ($p > 0.1$).

1. Introduction

Pregnancy can be complicated by three types of diabetes mellitus: type 1 (T1DM) and type 2 (T2DM) known as pregestational diabetes mellitus (PGDM) and gestational diabetes mellitus (GDM) [1–3]. Gestational diabetes mellitus is defined as a glucose intolerance resulting in hyperglycemia of variable severity with onset during pregnancy [1,3,4]. The prevalence of GDM is increasing worldwide, intensified with advancing maternal age, racial/ethnic disparities, obesity, and the diagnostic test used [5,6] Global prevalence of GDM ranges from 2 to 14% [7,8]. In Europe the prevalence of GDM is often reported as 2–6% of pregnancies [9]. In the United States, 7% of all pregnancies are complicated by GDM [1]. Especially high GDM prevalence (13–15%) was detected in ethnic minority women (South Asian, Chinese, Indian) in Australia [8,10]. GDM is associated with significantly more maternal and neonatal complications [5,9,10,11–13].

A hyperglycemic environment is associated with oxidative stress [5]. The role of oxidative stress in the onset, progression and complications of T1DM [14,15], T2DM [15,16] and GDM [17–19] has been described. These pathological pregnancies, including GDM, are associated with an increased levels of oxidative stress, due to overproduction of reactive oxygen species (ROS) and/or defects in antioxidant defenses [5]. Overproduction of ROS induces oxidative damage in membrane lipids, proteins and DNA, including purine and pyrimidyne bases and, as a consequence single strand breaks (SSBs), double-strand breaks (DSBs) and DNA–DNA or DNA-proteins cross-links [20–22].

Earlier studies with T1DM and T2DM patients used cytogenetic methods: chromosome aberrations (CA), micronucleus (MN) and sister chromatid exchange (SCE) assays as an indication for chromosome damage [23–27]. Witczak et al. [28] noted in an investigation with pregnant women with T1DM a significant increase of MN frequencies in these women vs. their newborns. To date, there have been no studies concerning CA, MN and SCE frequencies in pregnant women with GDM and in their newborns.

Sheth et al. [23] investigated the frequencies of SCE and CA in a group of 20 patients with T2DM. Mean values of SCE found in T2DM patients were higher in comparison to the controls had. No increased CA frequencies were found in the participants. Zuniga-Gonzalez et al.

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[24] observed significant increase of the MN frequency in 55 patients with T1DM. Cinklinic et al. [25] did not find a significant increase of the CA rate in peripheral blood lymphocytes of 35 adult patients with T1DM compared to the control group. Binci et al. [27] investigated the numbers of SCE and MN in a group of 50 adult patients with T2DM. The mean frequencies of MN and SCE were higher in this group compared to the controls.

The aim of the present study was to evaluate structural chromosome aberrations (CA) in pregnant women with GDM and in their newborns exposed *in utero* to hyperglycemia. The *in vitro* structural CA assay was used to evaluate in peripheral blood lymphocytes in mothers and in cord blood of their offspring.

2. Material and methods

2.1. Subjects

The approval of the Bioethics Committee of the Medical University of Lodz (RNN/121/13/KB, Feb. 16, 2013) was obtained for the collection of venous blood samples from mothers with GDM and those without DM and of umbilical cord blood samples from their newborns and for performing cytogenetic assays.

The study group included 35 pregnant mothers with gestational diabetes mellitus (GDM) and their 35 newborns. The control group included 30 pregnant mothers without diabetes mellitus (DM) and their 30 newborns. The positive control group comprised 10 pregnant healthy mothers without DM and their 10 newborns. GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations [29].

Prior to the pregnancy and in the 1st trimester the participants consumed 400 μg of folic acid daily to prevent neural tube defects. Lymphocytes were collected from peripheral blood of the mothers and from umbilical cord of the newborns. In the positive control group a known mutagen chlormethine hydrochloride (Sigma, St. Louis, MO, USA) (CAS 55–86-7) was added to the samples *in vitro* at a dose of 0.25 μg/mL. The chromosome aberration (CA) assay was performed as described by Preston et al. [30].

The deliveries took place at the Fetal-Maternal and Gynecology Department of Medical University of Lodz located in the Polish Mother's Memorial Hospital Research Institute in Lodz, Poland. Blood samples were collected from May 2013 to Oct. 2014. The clinical characteristics of the participants are listed in Table 1.

Table 1
Clinical characteristics of the participants.

	Mothers with GDM[a] (n = 35)	Mothers without DM[b] (n = 30)
Clinical data		
Age at delivery (years)	32. ± 3.8	30 ± 6.8
Height (cm)	165.0 ± 7.0	163.1 ± 8.5
Weight (kg)	69.5 ± 17	62.8 ± 13
BMI (kg/m^2) before pregnancy	25.5 ± 5.8	23.6 ± 4.7
Gestational weight gain (kg)	14.9 ± 3.8	14.1 ± 4.9
Smoking, n (%)	1(2.9%)	2 (6.7%)
Fasting glucose (mg/dL)	86.7 ± 10.9	80.5 ± 9.4
Mean HbA$_{1c}$[c] (%NGSP) 3rd trimester	5.2 ± 0.5	–
Mean HbA$_{1c}$ (mmol/molIFCC) 3rd trimester	33.6 ± 5.1	–
Mean HbA$_{1c}$ (mg/dLeAG) 3rd trimester	103.2 ± 13.3	–

	Newborns of mothers with GDM n = 35	Newborns of mothers without DM n = 30
Clinical data		
Gender (n) Female/ Male	18/17	15/15
Gestational Age (week)	37.2 ± 3.4	38.4 ± 2.5
Weight (g)	3260 ± 508	3204 ± 364
Length (cm)	55.0 ± 3.1	54.8 ± 2.8
Fasting glucose (mg/ dL)	61.5 ± 10.1	67 ± 18.0
Apgar points in 1 st minute	9 ± 1.1	9.5 ± 0.5
Apgar points in 5th minute	9 ± 0.9	10 ± 0.5
Age of fathers	34 ± 5	32 ± 7

[a] GDM- gestational diabetes mellitus.
[b] DM- diabetes mellitus.
[c] HbA$_{1C}$: glycated hemoglobin is a form of hemoglobin that is formed in a nonenzymatic glycation pathway by hemoglobin exposure to plasma glucose.

2.2. Lymphocyte macrocultures and microscopic evaluation

Blood samples (10 mL) were collected from mothers and umbilical cord blood samples (10 mL) from their newborns under sterile conditions immediately after separation of umbilical cord and placenta. Two cultures were set up from each mother and the corresponding newborn and harvested using standard procedures [30]. Lymphocyte cultures were prepared from heparinized peripheral and cord blood according to the method of Moorehead et al. [31]. For each assay, 10 mL peripheral and cord blood were allowed to sediment for 2–3 h. The culture medium was Eagle's fluid 1959 (MEM) (Biomed, Lublin, Poland) with added 10% (v/v) fetal calf serum (Biomed, Lublin, Poland) and with the antibiotics crystalline penicillin (100 IU/mL) and streptomycin (100 μg/mL) (Gibco, Grand Island, NY, USA). The blood cultures were incubated at 37 °C for 48 h. Phytohemagglutinin M (Gibco, Grand Island, NY, USA) was added to the culture medium at 0.1 mL/10 mL to stimulate cell divisions.

2.2.1. Chromosome aberration assay (CA)

CA assays was conducted according to standard procedures described by Preston et al. [30]. Metaphase plates were obtained from the macrocultures described above after 48 h [30]. Colcemid (Serva, Heidelberg, Germany) (0.15 μg/mL) was added to the culture 2 h prior to harvesting. Hypotonic shock was performed at room temperature with 0.075 M KCl (Serva, Heidelberg, Germany) for 5 min. The cells were centrifuged and Cornoy's fixative (methanol/acetic acid, 3:1, v/v) solution was freshly added and dropped on the slides. In the positive-controls, chlormethine hydrochloride of 0.25 μg/mL (Sigma, St. Louis, MO, USA) (CAS 55–86-7) was used. The slides were coded and stained with 5.0% Giemsa (pH 6.8) (Sigma, St. Louis, MO, USA) for 10 min and observed according to the standard procedure. Slides were evaluated with a light microscope (Leica Microsystems GmbH (Germany), model DM 2500) and computer with LAS EZ software (Leica Microsystems GmbH, Germany). Chromosome aberrations were classified according to the International System for Human Cytogenetic Nomenclature [32] and to the criteria published by Preston et al. [30]. In the study and the control groups 50–100 metaphases (46 chromosomes each) were analyzed per person.

2.3. Statistical analysis

Arithmetic mean and standard deviation (± SD) were calculated. The results were subjected to statistical analyses with STATISTICA 12 software (StatSoft, Poland). The Shapiro-Wilk test was used to determine the distribution. *U* Mann-Whitney test was used when two groups were compared depending on the type of distribution. The correlations of the values investigated in the study were estimated with Spearman's rank correlation test. The level of significance was determined (*p*). A *p*-value of less than 0.05 was considered as significant.

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