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The influence of maternal smoking on transferrin sialylation and fetal biometric parameters

Marta Wrześniak[a,*], Małgorzata Królik[b], Marta Kepinska[a], Halina Milnerowicz[a]

[a] Department of Biomedical and Environmental Analyses, Faculty of Pharmacy, Wroclaw Medical University, Borowska 211, 50-556 Wroclaw, Poland
[b] Early Pregnancy Pathology Clinic, Centre of Gynecology, Obstetrics and Neonatology, Reymonta 8, 45-066 Opole, Poland

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ABSTRACT

Objectives: Transferrin is a glycosylated protein responsible for transporting iron, an essential metal responsible for proper fetal development. Tobacco is a heavily used xenobiotic having a negative impact on the human body and pregnancy outcomes. Aims of this study was to examine the influence of tobacco smoking on transferrin sialic acid residues and their connection with fetal biometric parameters in women with iron-deficiency.
Methods: The study involved 173 samples from pregnant women, smokers and non-smokers, iron deficient and not. Transferrin sialylation was determined by capillary electrophoresis. The cadmium (Cd) level was measured by atomic absorption and the sialic acid concentration by the resorcinol method.
Results: Women with iron deficiencies who smoked gave birth earlier than non-smoking, non-iron-deficient women. The Cd level, but not the cotinine level, was positively correlated with transferrin sialylation in the blood of iron-deficient women who smoked; 3-, 4-, 5- and 6-sialoTf correlated negatively with fetal biometric parameters in the same group.
Conclusion: It has been shown the relationship between Cd from tobacco smoking and fetal biometric parameters observed only in the iron deficient group suggests an additive effect of these two factors, and indicate that mothers with anemia may be more susceptible to Cd toxicity and disturbed fetal development.

1. Introduction

Tobacco is one of the most widely used stimulants around the world. Even during pregnancy, 12% of women smoke despite the many adverse effect of this stimulant on pregnancy outcomes. Tobacco smoke consists of various components, including nicotine, carbon monoxide, nitric oxide and heavy metals, which increase the risk of infertility, ectopic pregnancy, miscarriage, spontaneous abortion, fetal malformations, intrauterine growth restriction (IUGR), fetal low birth weight (LBW) and preterm birth (Milnerowicz et al., 2000; Rogers, 2009; Agrawal et al., 2010).

Cadmium (Cd) is one of the components of tobacco smoke that affect pregnancy outcomes. This heavy metal has been proven to contribute to oxidative imbalance (Bizoń et al., 2011), aberrations in umbilical morphometry (Milnerowicz-Nabzdyk and Bizoń, 2015) leading to obstetric complications. Cd is also a recognized factor influencing fetal size. Fetal biometric parameters (FBP) correlated negatively with this heavy metal when mothers' urine Cd levels were above 1.5 μg/l (Kippler et al., 2012).

Moreover, Cd influences the metabolism of essential microelements such as copper, magnesium, selenium, zinc (Zn), and iron (Fe), which are crucial for proper fetal development (Milnerowicz and Chmarek, 2005). It is observed that women with low iron stores or an iron deficiency absorb more Cd (Satarug et al., 2004). In addition, there are reports suggesting that Cd induces the release of Fe from ferritin (Flora et al., 2008). Relevant to this study, this heavy metal binds to transferrin, an iron-transporting protein, changing its biochemical structure (Wang et al., 2016), which may impair iron passage and lead to fetal malnutrition.

Cd also influences the concentration of glycoproteins, as discovered in the study by Patel, et al. They observed a positive correlation, with a dose-related response, between α1-acid glycoprotein and the concentration of Cd in the blood of rats (Patel and Venkatakrishna-Bhatt, 1992). In addition, Cd affects the concentration of sialic acids (SA) in the human body. It causes the release of SA in the renal tubule and leads to a reduction of SA content in

* Corresponding author.
E-mail addresses: krolikm87@gmail.com (M. Wrześniak), halina.milnerowicz@umed.wroc.pl (H. Milnerowicz).

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erythrocytes. Finally, it raises the concentration of total sialic acids in the urine (Sillanaukee et al., 1999).

Transferrin (Tf) is a highly glycosylated protein. Transferrin has a high number of sialic acid residue variables. Shifts toward higher sialylated Tf isoforms are observed in pregnancy as well as in iron deficiency-related anemia (De Jong et al., 1995). Any improper iron level in the blood of pregnant women may lead to anemia and its related complications for the fetus, i.e. an increased risk of preterm delivery and LBW (Allen, 2000; Scholl and Reilly, 2000). There is report suggesting that higher sialylated Tf isoforms may facilitate Fe transport and delivery through the body (Hoefkens et al., 1997). While Tf sialic acids are supposed to be important in Fe distribution, any factor which may damage or improperly release sialic acids may indirectly influence Fe homeostasis.

Changes in the branching of glycans as well as in sialylation and galactosylation are observed after exposure to tobacco smoke (Knezevic et al., 2010). Furthermore, changes in the transferrin sialylation have been observed in the blood of smoking women after delivery a baby with IUGR (Wrześniak et al., 2015). Assuming that shifts in the number of Tf sialic acids residues have an influence on Fe transport to fetus, it is important to precisely measure the xenobiotics present in tobacco smoke causing this aberration.

The aim of the present study was to evaluate the influence of tobacco smoking on transferrin sialic acids residues and its association with FBP. In our study, we have selected anemic and non-anemic, but otherwise healthy pregnant women to investigate the potential way these xenobiotics influence the development of fetuses.

2. Material and methods

2.1. Subjects

Healthy pregnant women experiencing a proper course of pregnancy were qualified to participate in the study. Women's blood was collected three times during the pregnancy – in the 1st trimester, up to the 12th week of gestational age [GA]; the 2nd, from 13 to 26 weeks of GA; and the 3rd, from 27 weeks of GA, during regularly scheduled appointments with a physician. Gestational age was determined by the date of the last menstrual period and confirmed by ultrasound examination. Patients were divided into 4 groups based on incidence of tobacco smoking and iron deficiency: 1) smoking ID – smoking with iron deficiency (20 samples); smoking non-ID – smoking, non-iron-deficient (12 samples); non-smoking ID – non-smoking, iron-deficient (61 samples) and non-smoking non-ID – non-smoking, non-iron-deficient (80 samples) as a control group.

Iron-deficiency was diagnosed in women for whom two or more of the following parameters were observed: Fe concentration $\leq$50 μg/dl, total iron binding capacity (TIBC) $\geq$446 μg/dl, unsaturated iron binding capacity (UIBC) $\geq$346 μg/dl and transferrin saturation (TfS) $\leq$15% as an indicator of lowered iron stores. These parameters were determined in the serum of pregnant women in each trimester. Smoking status was self-reported and confirmed by measuring the concentration of cotinine, a nicotine metabolite, in the blood of the women.

The condition of fetuses was observed via ultrasound examination on an SSI 6000 SonoScape. The following fetal biometric parameters were collected: biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). Each FBP was divided by the week of pregnancy to eliminate any variability in fetal size associated with pregnancy duration and thus highlight the potential impact of smoking and iron deficiency on the size of the fetus. To determine the infant's condition, the Apgar score and infant birth weight were noted.

Venous blood samples were obtained via the standard procedure, using tubes with heparin for plasma (S-Monovette, Sarstedt, No: 04.1907) and with a clotting activator for serum (S-Monovette, Sarstedt, No: 04.1905) preparation. Serum and plasma samples were centrifuged 2500 × *g* for 17 min to separate the substances. Specimens were immediately frozen (−70 °C) until further use.

2.2. Exclusion criteria

Pregnant women with diabetes, insulin resistance, thyroid disease and ultimate miscarriage were excluded. Fetuses with IUGR, infection, malformations and genetic aberrations disqualified pregnant women from the study as well. Alcohol drinking disqualified patients from the study.

2.3. Ethical clearance

The study protocol was approved by the Local Bioethics Committee of Wroclaw Medical University (KB – 845/2012). Participants provided written consent to participate in this study.

2.4. Smoking status and Cd concentration

Smoking status was established by self-report via a questionnaire and verified by measuring the concentration of cotinine by a commercially available indirect immunoenzymatic method (Cotinine, LUCIO-Direct ELISA, No: 501.301). According to the manufacturer's instructions, all samples with cotinine level above 25 ng/ml were admitted to the smoking group.

Cd concentration was determined in the blood samples by graphite furnace atomic absorption using SOLAAR M6, Thermo Elemental Co at 228.8 nm wavelength with a Zeeman background correction. Reference materials (Recipe, BCR) were used to determine the calibration curve and controls.

2.5. Determination of transferrin concentration

To determine transferrin concentration, an immunoenzymatic test, the Human Transferrin ELISA Kit (AssayMax Human, AssayPro, No: ET2105-1), was used. Each serum sample was diluted prior to use (1:20000). Absorbance measurement was performed on a Multiskan Go (Thermo Scientific, Waltham, USA) at 450 nm wavelength.

2.6. Determination of Tf isoform distribution

The separation of Tf isoforms in the case of a number of SA residues was performed on the capillary electrophoresis (CE) system Beckman Coulter PA800plus, Pharmaceutical Analysis System. A 50 μm internal diameter fused-silica capillary with a total length of 40 cm was used with 10 cm to the separation window (No: P1310-004748), in addition to a CEofix CDT kit for Beckman Coulter (No: P1310-004760). Lyophilized serums (Level I, II CEofix CDT Control Serum, Analis, No: P1310-004769) were used as controls. After dissolving with redistilled water and mixing, they were treated as test samples. The analysis was carried out according to the manufacturer's instructions with modifications proposed by Lanz et al. (2004).

A detailed procedure is described in our previous paper (Wrześniak et al., 2015). In brief: after the installation of the capillary, conditioning (with 0.2 M NaOH) was performed. Before analyzing the specimen, each sample and control serum were saturated with an Fe solution in the following proportion: 60 μl of serum was added to a 60 μl Fe solution as proposed by Lanz et al. (2004). This change resulted in better sample resolution than the