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Hyperglycemia induces inflammatory mediators in the human chorionic villous

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ABSTRACT

This study was based on the hypothesis that IL-1β and its central regulator, the inflammasome, may play a role in the inflammatory condition exhibited by placental tissues from mothers with different gestational hyperglycemia levels. Pregnant women were classified according to the glycemic reference as non-diabetic (n = 15), mild gestational hyperglycemia (n = 15), gestational diabetes mellitus (n = 15) and type 2 diabetes mellitus (n = 15). We investigated levels of pro-inflammatory factors in maternal plasma and placental tissues (by ELISA or immunohistochemistry) and, NFKB activity (by electrophoretic mobility shift assay) and inflammasome protein expression (by Western blot) in chorionic villous. Maternal plasma and placental levels of inflammatory factors (IL-1β, IL-6, and MCP-1) were increased during all hyperglycemic conditions. Villous stroma cells showed strong immunoreactivity to CD68. In addition, with syncytiotrophoblast, the villous stroma cells were also stained to detect iNOS, MCP-1, TLR2, and TLR4. Although the levels of protein had fluctuated in the groups, NLRP1, NLRP3, ASC, and Caspase 1 were up-regulated in all hyperglycemic groups suggesting the inflammasome may be assembled in these pregnant women. The NFKB activity also exhibited higher levels in hyperglycemic groups, which might imply in pro-inflammatory cytokines production. In summary, increased maternal glucose levels during pregnancy changed systemic and placental inflammatory patterns, which occurred in parallel with the expression of inflammasome factors and processing and secretion of the pro-inflammatory cytokine IL-1β. These results suggest an inflammatory condition in all gestational hyperglycemic conditions, even in hyperglycemia that is less severe than gestational or overt diabetes, likely associated with inflammasome activation and inflammatory cytokine secretion. Inflammasome activation as a possible source of inflammatory factors may be an important target to be considered while managing hyperglycemia and preventing adverse pregnancy outcomes.

Abbreviations: IL-1β, interleukin-1β; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor alpha; IL-10, interleukin 10; iNOS, inducible nitric oxide synthase; TLR, Toll-like receptors; TLR2, Toll-like receptor 2; TLR4, Toll-like receptor 4; AIM2, interferon-inducible protein absent in melanoma 2; NLRC4, NLR family CARD domain containing 4; NLRs, Nod-like receptors; NLRP1, NLR family pyrin domain containing 1; NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis speck-like protein; NFKB, nuclear factor kappa-light-chain-enhancer of activated B cells; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular pattern molecules; EMSA, electrophoretic mobility shift assay; CARD, caspase activation and recruitment domain; DM1, type 1 diabetes mellitus; DM2, type 2 diabetes mellitus; GDM, Gestational diabetes mellitus; MGH, mild gestational hyperglycemia; GM, glycemic mean; GP, glucose profile; GTT, glucose tolerance test

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1. Introduction

Pregnancies complicated by hyperglycemia are associated with short-term and long-term risk of adverse outcomes in both mother and offspring, which includes disturbances in embryonic and fetal development, type 2 diabetes mellitus (DM2), and cardiovascular diseases [1–4]. Implications for adverse intra-uterine programming have also been largely discussed in the literature [5]. These complications are not restricted to pregnancies associated with pre-gestational (type 1 diabetes mellitus, DM1, or DM2) or gestational diabetes (GDM). It also occurs in pregnant women diagnosed with mild gestational hyperglycemia (MGH), a condition in which the pregnant woman has a normal glucose tolerance test, but altered glycemic profile [5,6]. Increased rates of perinatal mortality (tenfold compared with non-diabetic pregnant women) and augmented incidence of infants born large-for-gestational-age or with macrosomia (rates similar to those seen in diabetic pregnant women [2,7]) and, incidence of type 2 diabetes (DM2) after pregnancy equivalent to GDM have been reported in pregnant women with MGH [8].

Changes in the maternal environment have received particular attention in the last decades as they can program permanent effects on the fetal physiology and metabolism. This fetal programming may result from different stimuli throughout pregnancy or at specific periods and increases the prevalence of coronary heart disease and diabetes, among many other conditions during adulthood [9]. Metabolic adaptations to fail or excess the fetal nutritional demand (as for glucose in diabetic pregnancies), stress, chronic and acute inflammation are some of the aspects that are being studied [10]. The unbalanced expression of inflammatory mediators alter the maternal environment and metabolism during gestation and increase the potential for gestational systemic insulin resistance, participating in the development of DM2 and GDM [11–12]. Inflammation at the placental territory also contributes to the fetal developmental environment and therefore has been the subject of numerous studies in hyperglycemic gestations [9].

During diabetes, high levels of serum glucose are also associated with increased levels of lipid serum [11] and both induce the expression of Toll-like receptors (TLR) 2 and TLR4 in macrophages and placenta [13]. These receptors recognize conserved pathogens-associated molecular patterns (PAMPs), inducing innate immune responses which are essential for host defenses [14]. They can also be activated by endogenous molecules (damage-associated molecular patterns, DAMPs) released under conditions of cellular stress or tissue injury [15] as free fatty acids and glycation end products [16]. TLRs and nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) trigger the formation of an inflammasome in the cytoplasm, a multiprotein complex, when active, recruits inactive procaspase-1 molecules to form activated caspase-1 [17].

The assembly of the inflammasome complex requires a particular NLR family protein (or AIM2), the Apoptosis-associated Speck-like protein containing a CARD (ASC) adaptor protein and pro-caspases-1, 5 and 8 [17]. Among the known inflammasome complexes (NLRP1, NLRP3, NLRC4, and AIM2) the best-characterized inflammasome is NLRP3, whose activation is thought to be dependent on NFKB signaling [18].

Caspase-1–mediated cleavage activates the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 into their secreted active forms [13,17]. IL-1β acts as a signal cytokine of the local injury and is involved in secondary cascades for the production and release of additional pro-inflammatory cytokine and therefore influencing local and systemic immune responses to resolve the injury-triggering factor. The mechanisms associated with the inflammasome in diabetes have been widely discussed in the literature [19], but little is known about gestational hyperglycemia that is not characterized as DM2 or GDM.

During gestation, the maternal blood flows into the placental intervillous space, allowing the exchange of oxygen and nutrients between fetal and maternal organisms and exposing the maternal blood microenvironment to the fetal component of the placenta, the chorionic villi [20]. Changes in this environment, such as increased glucose levels, whether enough to induce inflammasome activity and consequently inflammatory cytokines production may, therefore, affect gestation and impact the development and adult life of the neonate.

In this context, we herein compare the levels of pro-inflammatory factors in serum and fetal placental compartment (chorionic villous) in different hyperglycemic gestational conditions (mild gestational hyperglycemia, gestational diabetes mellitus, and type 2 diabetes). In addition, we analyze the expression of inflammasome proteins in order to understand whether it may have a potential role in the subclinical inflammatory condition that is also seen in the mild gestational hyperglycemia.

2. Methods

2.1. Study design and subjects

The Research Ethics Committee on human beings from Botucatu Medical School, UNESP, Brazil have approved all the procedures used in this cross-sectional study. Written informed consent was obtained from all subjects according to the principles of the Declaration of Helsinki.

Sixty pregnant women, allocated in four study groups, participated in the study. Pregnant women with DM2 (n = 15) were referred to the Diabetes and Pregnancy Service of Botucatu Medical School with a confirmed diagnosis. The diagnosis of GDMor MGHwas established between 24 and 28th gestational weeks, by the 75-g glucose tolerance test (75 g-GTT) according to ADA's criteria [26] and/or the glucose profile (GP - fasting glucose ≥90 mg/dL or postprandial glucose ≥130 mg/dL) test according to Rudge [6]. From the data, the women were classified into the following study groups: non-diabetic (ND; normal 75-g GTT and GP; n = 15), MGH (n = 15; normal 75-g GTT and abnormal GP) GDM (abnormal 75-g GTT first reported during the pregnancy; n = 15).

According to our treatment protocol, the glycemic control was achieved with fasting glucose < 95 mg/dL, 1 h post-prandial < 140 mg/dL, and 2 h post-prandial < 120 mg/dl. At this time, MGH and GDM pregnant women were initially treated with diet and exercise; insulinwasadministered only if necessary. All DM2 pregnant women were treated with diet, exercise and insulin at the first attendance in our service, and were readjusted weekly [6,7,12].

2.2. Exclusion criteria and subject characterization

The exclusion criterion for this study was as follows: women with DM1, multiple pregnancies, fetal malformations, deliveries before the 37th week, self-reported as current daily smokers, active or past infection with hepatitis C, hepatitis B, HIV or tuberculosis, family history of cancer, diagnosed coronary artery disease or females who are under treatment for a medical condition requiring chronic use of medications that were unrelated to the DM2 condition [6,7].

Pregnant women in this study were characterized by age, weight gain during pregnancy, glycated hemoglobin levels (HbA1c) during the third trimester (high-performance liquid chromatography—D10™ Hemoglobin Testing System, Bio-Rad Laboratories, Hercules, USA) and glycemic mean (GM). For the pregnant women classified as DM2, GDM, and MGH, the GM was calculated by the arithmetic mean of all plasma glycemic levels evaluated in all GP performed during pregnancy. For the ND pregnant women, the GM was calculated by the arithmetic mean of all plasma glycemic levels assessed in the diagnostic GP. All plasma glucose levels were evaluated by the glucose oxidase method (Glucose Analyzer II Beckman®, Fullerton, CA, USA) [6,7].