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Visfatin gene expression and oxidative stress in pregnancy induced hypertension

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ABSTRACT

Preeclampsia, a hypertensive disorder of pregnancy, was related to hypertension, diabetes, oxidative stress, obesity, in addition to polycystic ovarian diseases. Visfatin, potentially a new adipokine has emerged having high contribution in pathogenesis of preeclampsia. Oxidative stress was increased lipid peroxidation and caused vascular endothelial damage. This study was conducted with the aim of evaluating the level of visfatin gene expression in placenta and measuring some oxidative stress parameters. Eighty Egyptian patients of newly diagnosed pregnant hypertensive disorder were selected for the study which was recruited from Mansoura University Hospital, Department of Obstetrics and Gynecology and also normotensive pregnant women were collected. The pregnant women groups were classified into four groups: gestational hypertension (n = 20), mild preeclampsia (n = 16), severe preeclampsia (n = 25), chronic hypertension with superimposed preeclampsia (n = 19) and compared with normotensive pregnant women (n = 10) as control group. Visfatin gene expression level was decreased in placenta of pregnant hypertensive disorder women groups with mean 1.28 ± 0.42, 1.01 ± 0.24, 0.40 ± 0.14 and 0.32 ± 0.11 respectively when compared to normotensive pregnant women with mean 1.56 ± 0.69. Additionally, catalase activity, total antioxidant capacity and reduced glutathione levels were decreased in hypertensive pregnant women groups compared with normotensive ones. On the other hand malondialdhyde level was increased in preeclampsia groups when compared with normalized pregnant women. Decreased visfatin level has an important pathophysiology of preeclampsia and suggests the complication in pregnancy. Also the imbalance between oxidant and antioxidant has an important causative factor in the pathogenesis of preeclampsia.

Introduction

A condition in pregnancy characterized by high blood pressure contributes greatly to maternal morbidity and mortality and the fetus as well around the world [1]. The mere solution for preeclampsia and pregnancy induced hypertension is delivery of the fetus and placenta [2]. Blood pressure's interruptions that happen in pregnancy have harmful influence on organ systems of both the pregnant woman and the fetus [3]. Complications in pregnant women suffer from preeclampsia are seizure activity, placental abruption, stroke, hemolysis, hemolysis elevated liver enzymes and low platelets (HELLP) syndrome, liver hemorrhage, pulmonary edema, acute renal failure, and disseminated intravascular coagulation [4].

The exact reasons of preeclampsia are not well known so risk of it may be elevated by some factors such as the first pregnancy [5]. Also women with a history of preeclampsia [4] and multiple gestations elevate the risk [2]. Furthermore, some disease present before pregnancy such as obesity, diabetes mellitus, insulin resistance, chronic hypertension, gestational diabetes, lupus, vascular or connective tissue disorders and also chronic kidney disease may be increase the risk of preeclampsia [6]. High blood pressure during pregnancy is divided into four groups as recommended by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. The first group is chronic hypertension, the second group is gestational hypertension, the third group is mild and severe preeclampsia and the fourth group is preeclampsia superimposed on chronic hypertension [7]. Gestational hypertension and preeclampsia generally can be diagnosed by measurements of high blood pressure and proteinuria [8]. Also some symptoms may be associated with increasing in blood

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pressure such as vision interruption, continuous acute headaches, unexpected expand in face, hands and feet, vomiting, epigastric pain, lowering in platelets and increasing in liver enzymes and serum creatinine [9].

Adipokines play an important role in some process including inflammation, organization of food intake, and arrangement of body weight homeostasis, proliferation, insulin sensitivity, immunity and vascular homeostasis [10]. In obesity and type 2 diabetes mellitus have an alteration in adipokine production. Also, there is imbalance in adipokine production at the onset of insulin resistance, adipose tissue inflammation, chronic systemic inflammation, cardiovascular disease and endothelial dysfunction [11]. Fukuhara et al. [12] are the first one that characterize visfatin as an adipokine and show that insulin mimetic properties in mice with binding to and promoting the insulin receptor. Visfatin was found identical to pre-B cell colony enhancing factor (PBEF). It is a highly conserved 52 kDa cytokine-like protein. It increases the maturation of B cell precursors in relation to interleukin-7 and stem cell factor [11] and also suppresses the apoptosis of neutrophils [13]. Rongvaux et al. [14] reported that visfatin displays intrinsic enzymatic activity as a nicotinamide phosphoribosyl transferase (Nampt). However, the physiological connection of NAMPT stay argumentative [15], Revollo et al. [16] showed that the critical role of NAMPT in the monitoring of glucose metabolism through the NAD biosynthetic activity.

Pathogenesis of preeclampsia is influenced by oxidative stress. It could lead to tissue damage by the end [17]. An adaptive mechanism promoting the antioxidant defense system in pregnant women to oppose the effect of oxygen active species through enzymatic antioxidants like superoxide dismutase, glutathione peroxidase, catalase and also non-enzymatic antioxidants like reduced glutathione can hinder the occurrence of oxidative stress in preeclampsia [18]. Elevation of lipid peroxidation products causes impaired antioxidant enzyme defense mechanism and this imbalance may result in preeclampsia pathogenesis [19].

The purpose of this study was to evaluate the visfatin gene expression level in placental hypertensive and normotensive pregnant women groups as well as some antioxidants were determined in all groups.

Subjects and methods

Patients

The study was conducted from May 2014 until December 2016; patients chosen from the Obstetrics and Gynecology Department, Mansoura University. The study group was comprised 80 Egyptian pregnant women with hypertensive disorder and was divided into four groups. Group I (gestational hypertensive): twenty of pregnant women were gestational hypertensive with the age range 27 to 41 years with the mean age 34.40 ± 4.27 years which defined as blood pressure $\geq$ 140/90 mmHg after gestational age 20 weeks without proteinuria. Group II (mild preeclampsia): Sixteen of pregnant women were mild preeclampsia with the age range 33 to 46 years with the mean age 39.63 ± 4.40 years which defined as BP $\geq$ 140/90 mmHg after gestational age 20 weeks with significant proteinuria (+ protein in urine on dipstick). Group III (severe preeclampsia): Twenty-five of pregnant women were severe preeclampsia with the age range 21 to 40 years with the mean age 30.28 ± 6.39 years. Severe preeclampsia was determined with blood pressure $\geq$ 160/110 mmHg with excessive proteinuria ($\geq$ + +++ protein in urine on dipstick). Group IV (chronic hypertension with superimposed preeclampsia): Nineteen of pregnant women were chronic hypertension with superimposed preeclampsia with the age range 28 to 45 years with the mean age 36.84 ± 5.40 years. The preeclampsia with hypertension present before pregnancy was defined as certified BP $\geq$ 140/90 prior to pregnancy with new onset proteinuria (+ protein in urine on dipstick). Group V (Normotensive group): The normotensive pregnant women comprised 10 with the age range 21 to 42 years with the mean age 31.20 ± 7.79 years. Normal participants were at the same maternal and gestational age. Also, their pre-pregnancy parity and maternal body mass index were equal.

An informed written approval was provided by all study participants. Additionally, the Ethical Board of Mansoura University approved conducting the study. Also, the study was delimited to patients who did not provide a written approval, those who smoke, those with gestational diabetes, infectious disease, and premature rupture of membrane or suffer from other medical diseases. Moreover, participants with abnormal glucose tolerance who participated in the gestation test administration at weeks 24–28 or suffer from other medical diseases were also eliminated.

Tissue preparation

Placental biopsy samples were obtained during Caesarean sections from both normotensive patients and those with hypertensive pregnant women. Only some placental samples taken from a woman by a caesarean operation were included in the study to prevent any probable effects of labor on visfatin gene expressions. An anatomization of a central area of chronic tissue and extraction of maternal deciduas and amnionic membranes were taken for analysis. Large tissue samples cut to $\leq$ 0.5 cm in any single dimension then placed the fresh tissue in in epindorff containing 500 μl of RNA later tissue collection (RNA later Inc., Austin, Texas, U.S.). Tissue samples in RNA later solution were stored at 4 °C for 2 week without compromising RNA quality.

Blood collection

Morning fasting blood samples were collected from the study participants to be estimated after applying all aseptic precautions. One ml blood was collected in EDTA tubes for estimation of reduced glutathione level and the rest blood was collected into clean and dry test tubes then allowed to clot, serum was separated by centrifugation at 3000 rpm for 10 min and then stored in deep frozen at −20 °C until used. Serum was used for estimation of catalase activity, total antioxidant capacity level and malondialdhyde level.

RNA isolation and quantitative PCR

Total RNA was collected from placental tissue samples utilizing vivantis kit (Vivantis Technologies Sdn. Bhd., Malaysia), based on the manufacturer's instructions. The extracted RNA was transcript into cDNA by using seni FAST™ cDNA synthesis kit (Bioline USA Inc., USA), based on the manufacturer's instructions. The housekeeping gene (GAPDH) was used to normalize mRNA concentrations. Quantitative PCR was performed for comparing expression levels of visfatin transcripts. The relative expression levels of visfatin in the tissue samples and reference sample were assessed by quantitative PCR using real-time RT–PCR analyses (Piko Real 96 type 5100, thermo scientific). RT–PCRs were performed using a seni FAST™ SYBR NO-ROX kit (Bioline USA Inc., USA) in a final volume of 20 ml. An initial PCR activation step was 2 min at 95 °C followed by 40 cycles of 5 s at 95 °C, and 20 s at 60 °C and 10 s at 72 °C. The sequence of primers, used in real-time PCR, was designed according to Vivantis Technologies Sdn. Bhd., Malaysia to span introns to prevent detection of genomic DNA as shown in

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