The Relationship Between Maternal Serum Visfatin Level And Hypertensive Disorders of Pregnancy

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Citation

Abstract

Background and Objective: Hypertensive disorders of pregnancy are amongst the most common conditions adversely affecting maternal and fetal prognosis, leading to noticeably increased morbidity and mortality in the prenatal period. Pre-eclampsia (PE), in particular, is considered as a cardiovascular complication, having risk factors in common with metabolic syndrome of which insulin resistance and obesity are a part. Visfatin is a recently introduced adipokine with insulin-mimetic properties which is thought to contribute to the components of metabolic syndrome. There have been notions supporting the relationship between level alterations of this novel adipokine and affliction of pregnant women with PE. The current study aims to evaluate the correlation between visfatin quantitative levels and the presence of pregnancy hypertensive disorders including mild PE, severe PE and chronic hypertension (HTN). Moreover, assessment of normal pregnancy visfatin levels as the control is done and the data are compared with those of pregnancies with hypertensive disorders. Materials and methods: This cross-sectional study which recruited cases of mild PE (N=40), severe PE (N=40), chronic HTN (N=40) also included normal pregnant subjects as control (N=60). The cases were of the patients hospitalized at university affiliated centers and the controls consisted of referrals to the outpatient departments (OPD). The subjects were matched for Body Mass Index (BMI), parity, gestational age; they were excluded in case they had gestational diabetes, infectious disease, premature rupture of membrane (PROM), any other medical disease, or in labor phase. Informed consents were signed by all the participants. Then, their fasting blood samples were drawn, kept at -20°C. Enzyme Linked Immune Sorbent Assay (ELIZA) test was performed to determine visfatin level. The data were then analyzed, using Mann-Whitney and Krus-Kal-Wallis statistical methods. Results: The mean of serum level for visfatin was 2.81±0.37ng/ml (mean±SEM) for the pregnant control group while it was 2.66±0.41, 7.99±0.28, and 3.46±0.66ng/ml for the mild PE, severe PE and chronic hypertension cases, respectively. Based on the mean values for circulating visfatin level amongst the groups, visfatin level was significantly higher in the severe PE group as compared with the control (p<.0001), mild PE (p<.0001) and chronic HTN (p<.0001) with correspondent mean differences of 5.1, 5.3 and 4.5, being significant at the 0.05 level. Comparison of the mean visfatin levels of mild PE (P=0.79) and chronic HTN (P=0.29) with the controls revealed no significant difference. Conclusions: Severe PE cases were shown to represent higher circulating visfatin levels compared to the normal pregnant women, mild PE and chronic HTN cases. This may further suggest the potential role of visfatin in pathogenesis of severe PE during pregnancy, leading to proposal of models in prognosis definition, prevention and possibly management of this condition through visfatin level related interventions in the future.

ABBREVIATIONS
BMI: Body Mass Index
BP: Blood Pressure
DM: Diabetes Mellitus
ELIZA: Enzyme Linked Immune Sorbent Assay
GDM: Gestational Diabetes Mellitus
GA: Gestational Age
HTN: Hypertension
IUGR: Intra Uterine Growth Retardation
OPD: Out Patient Department
PE: Pre-eclampsia
PROM: Premature Rupture Of Membrane
SEM: Standard Error of Mean
TNF-α: Tumor Necrosis Factor- alfa
Wt: Weight

INTRODUCTION
Hypertensive disorders of pregnancy including (but not limited to) pre-eclampsia (PE) and chronic hypertension are characterized as prevalent disorders during pregnancy which potentially lead to remarkable prenatal morbidity and are considered as one of three causes of death in pregnant
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women (1-4).

PE (being defined as increased blood pressure equal to or above 140/90 mmHg in the presence of proteinuria) and chronic HTN, have not been fully elucidated despite tremendous efforts so far in terms of their etiologic contributors, pathogenesis and other yet to find features (3-5).

To clinically define PE as stated above, patients should have fulfilled criteria as BP≥140/90 after gestational age of 20 weeks, plus proteinuria equal to or more than 300mg in 24 hour urine or more than 1+ in dipstick urine test (4, 5). Some indicators are specific for definition of severe PE including BP≥160/110 mmHg, 2+ or more proteinuria, headache and/or visual disturbance, epigastric pain, oliguria, elevated plasma creatinin, low platelet and elevated liver enzymes (3-5).

Chronic hypertension is defined as documented BP≥140/90 prior to pregnancy or diagnosed not later than the week 20 of the gestation (5).

There are a variety of contributing factors in pathophysiology of pre-eclampsia of which, alteration in the serum levels of adipokines such as adiponectin, resistin and leptins has been widely pointed out in the literature (1, 2, 4, 7, 8).

Considering the extent of insulin resistance during pregnancy, the second half is the period when this state is prominent. Consequently, preeclampsia which is an exaggerated format of insulin resistance becomes more evident. Other element such as leptin has been proved to be increased during pregnancy and more significantly when PE occurs (6). Adiponectin is observed contradictorily to decrease or remain steady during normal pregnancy and decrease, increase or remain fixed in PE in different studies (1, 2, 4, 6, 8).

Pregnancy period is when redistribution of adipose tissue occurs. This adipose tissue is a metabolically active tissue rather than just a storage depot. It produces adipocytokines exerting endo-paracrine effects (21). Adipocytokines including leptin and adiponectin play a role in insulin resistance and homeostasis of energy; therefore, alterations contribute to metabolic conditions (12-15).

In addition to these adipocytokines, some newer elements are isolated (1,2). Visfatin is a newly introduced adipokine which is mainly secreted by the visceral adipose tissue rather than by subcutaneous fat. This novel adipokine is suggested to improve glucose tolerance, playing a role in development of obesity associated insulin resistance and diabetes mellitus (DM) type II (12, 13, 15, 16).

Further works done on visfatin have suggested that this adipokine is also a pre-B cell colony-enhancing element which mimics insulin effects binding to insulin receptor although on a different site than insulin itself (10, 11, 17, 18). Following receptor binding, visfatin activates the insulin signal transduction cascade enhancing glucose transport in the muscle and adipose tissue and diminishes glucose delivery to tissues by hepatocytes (13, 14, 16).

There have been a number of researches evaluating the correlation between leptin, Tumor Necrosis Factor Alfa (TNFα) and adiponectin level, and presence of PE in pregnancy (6-8, 13, 21); however, despite the two studies one by Wensheng et al. (2) reporting decreased vasfatin level in PE and the other by Fasshauer et al. (1) indicating increased visfatin level in PE, such comprehensive works on the level of “insulin mimetic visfatin” in pregnancies with PE are by far lacking.

Based on the available data on the effect of alterations in the serum levels of leptin, adiponectin and resistin plus the literature-based notions indicating that changes in the circulating visfatin levels have been coincidental with insulin resistance associated diseases such as type 2 DM, gestational diabetes and obesity (namely metabolic syndrome) (9-12, 16, 18, 20, 21), and considering the physiological characteristics and activity of visfatin (17), we arrived at the hypothesis that the circulating visfatin level is altered in women with PE. In order to verify this hypothesis, we planned to conduct this investigation to determine the quality of variations in the serum level of visfatin in conditions like mild PE, severe PE and chronic HTN; there was also an attempt to compare the values with those of the normal pregnant control subjects.

MATERIALS AND METHODS

In this cross-sectional study, we recruited a total number of 180 subjects from whom 120 participants were cases of hypertensive disorders of pregnancy and 60 were normal pregnant women as controls. The cases were subdivided into three separate groups comprising 40 cases of mild preeclampsia, 40 cases of severe preeclampsia, and 40 cases of chronic hypertension. All the cases and controls were evenly matched and balanced for parity, BMI and gestational age. Exclusion criteria were gestational diabetes, infectious disease, premature rupture of membrane, any other medical
disease and being in labor phase. We also excluded those cases with abnormal glucose tolerance test conducted at weeks 24-28 of gestation. Control pregnant cases were selected if they were healthy reproductive women with none of aforementioned exclusion criteria.

Inclusion of cases in each subgroup including mild PE, severe PE and chronic hypertension was carefully done in compliance with the following definitions. Preeclampsia: blood pressure $\geq 140/90$ mmHg in two occasions at least 6 hours apart, detected after week 20 of gestation, in combination with proteinuria ($\geq 1+$ on dipstick urine test or $\geq 300$mg or more in 24 hour urine sample). In patients who were diagnosed and categorized as severe preeclampsia, one or more of the followings were evident: BP$\geq 160/110$mmHg, excretion of $\geq 500$mg protein in 24 hour urine sample or 3+ or 4+ proteinuria on dipstick and/or presence of oliguria, pulmonary edema, cerebral or visual disturbances, epigastric or right upper quadrant abdominal pain (27).

The cases were selected from those patients hospitalized at university affiliated centers and the controls were of those referring to OPD clinics from December 2008 to February 2010. All participants were asked to provide informed written consent. The work was approved by the ethics committee of the institution.

Fasting blood samples were drawn from all the subjects. The collected serums were then preserved in aliquot at $\leq -20^\circ C$ prior to assay. Enzyme linked immune sorbent assay (ELIZA) was the method applied to determine visfatin concentration levels (ng/ml). The assay was conducted using Biovision Human visfatin ELIZA kit (Biovision Research Products- CA94043. USA) and according to the manufacturer's instructions. With detection limit of 0.1ng/ml, the kit measurability for visfatin ranged from 0.125 to 8ng/ml.

The obtained data were then statistically analyzed through Mann-Whitney and Krus. Kal-Wallis methods exerting mean $\pm$ SEM for visfatin concentration in each group. The cases and controls were followed until after delivery at term gestation when birth weight and its correlation with visfatin level were also assessed. Student t-test and one way ANOVA were applied to compare the means between and among groups respectively. When ANOVA was significant, post- hoc LSD test was performed to compare the data between the groups. Pearson linear correlation was also used to further evaluate whether quantitative variables had a linear correlation. The analysis was done using SPSS software version 11.5. The probability value of less than 0.05 was considered significant.

RESULTS
Mean age was 29.2±4.9 (ranged 20 to 43), while mean BMI was 29.1±2.9 (ranged 23.4 to 37.6). Mean age and BMI were not significantly different between four groups. (Table I)

The mean serum level of visfatin for pregnant control group, mild PE, severe PE and chronic HTN were 2.82/2.04, 2.66/1.84, 7.99/1.29 and 3.46/2.68 ng/ml respectively. Mean levels for serum visfatin were significantly higher in severe PE group compared to control, mild PE and chronic HTN (P<0.001, Table II).

The other index we evaluated its correlation with circulating visfatin level during pregnancy, was the birth weight. All participants were followed up until after term delivery when neonatal weight were measured and compared amongst groups, after being matched for gestational age.

The mean birth weight differences were more pronounced in group 3 where P value denotes marked significance and univariate pearson correlation test indicates the negative linear correlation between visfatin level and neonatal weight. ($r=-0.597$, $P<0.001$, Figure I)
**DISCUSSION**

In the current study, the data obtained supported the influence of PE severity on the circulating visfatin level. These findings for the first time demonstrated that up regulation of visfatin level is correlated with severe PE rather than the controls. However, this failed to prove a significant correlation with conditions like mild PE and chronic HTN as compared to controls.
Furthermore, we arrived at novel findings showing negative linear correlation between visfatin and neonatal weight. Lower neonatal weight was more abundantly seen among the cases with high visfatin levels.

Hyperinsulinemia, glucose intolerance and lipid abnormalities are evident in the second half of pregnancy (12-14), where adipokines including adiponectins, resistin and leptin have been reported to reach to almost two fold higher levels during pregnancy than the non-pregnancy state (21-23). There have been numerous works which contradictorily report the adipokines subjected to increase (22, 25), decrease (24) and no change (26) in normal pregnant women compared to non-pregnant ones.

Other than hypertensive disorders of pregnancy, visfatin may play a role in the pathogenesis of conditions like gestational diabetes mellitus (GDM) (27), intrauterine growth retardation (IUGR) (1) and severity of disease burden in chronic kidney disease during pregnancy (18).

In an investigation by Krzyzanowska, et al. adipokines, namely visfatin, were shown to have 1.4 fold increased concentration in women with GDM as compared to the gestational age matched controls (27). This in part shows a possible negative correlation of visfatin level with insulin resistance index which was also shown in a separate study conducted by Fasshauer M. et al (1). They also revealed that fasting insulin levels are in negative correlation with circulating visfatin in GDM. This may signify the role of visfatin in intensifying the symptoms of GDM patients; however, the mechanism by which visfatin is increased over the course of GDM is yet to be elucidated. Furthermore, Fasshauer M. et al. demonstrated in a recent study that during the third trimester, in patients with IUGR, visfatin is about two fold increased as compared to healthy pregnant women (1). Similarly, Axelsson J. et al. showed that, higher plasma visfatin levels predict higher mortality in patients who suffer from chronic kidney disease in pregnancy after being matched for age and sex (18).

To demonstrate the correlation of visfatin level with hypertensive disorders of pregnancy, the number of performed studies is scant. Specifications and outcomes of two main cross-sectional controlled studies along with the current investigation are outlined in Table III.

In the current study, we compared the mean values of visfatin as a novel adipokine in normal pregnant controls with its circulating levels among mild PE, severe PE and chronic hypertension cases. Partly in agreement with our findings was the study by Fasshauer et al (1), denoting the increased levels of visfatin in PE; however, that investigation did not assess visfatin level in severe PE and chronic HTN as other components of hypertensive disorders of pregnancy.

In contrast to our findings, Wensheng Hu et al. (2) reported a decrease in visfatin level in PE compared to controls. Our study was the first research of a kind evaluating visfatin level in hypertensive disorders of pregnancy when segmented into mild PE, severe PE and chronic HTN. Neonatal weight correlation with circulating visfatin level among hypertensive (mild PE, severe PE and chronic HTN) groups and controls was for the first time considered here as a sub-analysis. Due to limitations we faced while conducting a cross-sectional survey, causal relationship between visfatin level increase and pathogenesis of severe PE could not be assessed.

A hypothesis which is worth being investigated is that increased synthesis or decreased degradation of visfatin in PE might contribute to named conditions. To our knowledge expression of visfatin has not been yet determined in the placental tissue; however, further studies including more variables in different settings are required to verify this subject. It is believed that the primary findings of this study can motivate conduction of further comprehensive investigations in the field of adipokines and hypertensive disorders of pregnancy.

**CONCLUSIONS**

In summary; based on the evident data on the correlation between adipokines and PE and what we reported regarding higher visfatin levels in pregnancies with severe PE compared to normal pregnancies, the results may further suggest the potential role of visfatin in the pathogenesis of severe PE during pregnancy. This may lead to the proposal of models on predictive prognostic value of visfatin which help prevention or possibly management of severe PE through visfatin level related interventions in the future.

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