Plasma Fibronectin Concentration in Obese/Overweight Pregnant Women: A Possible Risk Factor for Preeclampsia

Itemobong S. Ekaidem · Debayo M. Bolarin ·
Alphonsus E. Udoh · Saturday J. Etuk ·
Christopher E. J. Udiog

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Abstract Plasma fibronectin (FN) levels in obese/overweight and non-obese pregnant women were evaluated as a possible risk factor for preeclampsia. A total of one hundred and sixty three pregnant women attending antenatal clinic at University of Calabar Teaching Hospital participated in the study and sixty non-pregnant women served as control. About 77 (47.24%) of the pregnant women were followed up for any subsequent development of preeclampsia during the pregnancy. Fibronectin levels in plasma were measured by ELISA assay and serum total protein, urea and creatinine were determined spectrophotometrically. The mean plasma FN concentration of non-obese pregnant women in first trimester was lower than those of the non-pregnant women by 24%, but however, increased to the non-pregnant level in second and third trimesters. Obese/overweight pregnant women had significantly ($P < 0.05$) higher values than non-obese pregnant women in second and third trimesters. FN in obese/overweight pregnant women correlated positively with mean arterial blood pressure (MAP: $r = 0.414$, $P = 0.04$). About 28.57% of the pregnant women with FN above cut off point of 330 $\mu\text{g/ml}$ at 18–24 weeks of gestation developed preeclampsia. This value increased to 40.0% when only the

obese/overweight women were considered. On analysis of both fibronectin $>330 \mu\text{g/ml}$ and MAP > 90 , the predictive value increased to 66.7%. We therefore conclude that elevated FN may be regarded as a risk factor of preeclampsia especially among the obese women.

Keywords Fibronectin · Obesity · Arterial pressure · Risk factor · Pregnancy · Preeclampsia

Introduction

Fibronectins are large dimeric connective tissue glycoproteins of 450 kDa molecular weight. They are present in the extracellular matrices and cell surfaces as insoluble proteins and in plasma as soluble proteins. The soluble fibronectins are synthesized by hepatocytes while the insoluble forms are produced by fibroblasts, macrophages, endothelial cells and epithelial cells.

Fibronectins promote cell–cell and cell–matrix interactions and thus play important role in tissue construction and reconstruction during embryogenesis and wound healing. They provide positional cues to direct varieties of biological processes including cell migration, adhesion, differentiation and apoptosis [1]. Changes in the concentration of fibronectin in tissues and body fluids have been reported in various disease conditions. For example elevated levels of fibronectin have been found in plasma of patients with rheumatoid vasculitis [2], collagen vascular diseases, diabetes mellitus [3], chronic obstructive pulmonary disease [4], ischemic heart disease [5], renal cancer [6], gastrointestinal cancer [7], acute myocardial infarction [8], coronary artery disease [9] and trauma [10]. Increased levels of fibronectin in plasma of patients with some of these conditions were attributed to increased synthesis caused by

I. S. Ekaidem (✉)
Department of Chemical Pathology, Faculty of Clinical
Sciences, College of Health Science, University of Uyo,
P.M.B. 1017, Uyo, Nigeria
e-mail: seityjen@yahoo.com

D. M. Bolarin · A. E. Udoh · C. E. J. Udiog
Department of Chemical Pathology, College of Medical
Sciences, University of Calabar, Calabar, Nigeria

S. J. Etuk
Department of Obstetrics and Gynaecology, College of Medical
Sciences, University of Calabar, Calabar, Nigeria

proinflammatory cytokines, and release of extracellular matrix and cell surface fibronectins into circulating plasma [3].

The pathogenesis of preeclampsia is not well understood, but genetic and immunological factors have been considered. The mechanism of production of clinical disease stems from altered maternal response to the invading trophoblast, leading to failure of the second wave of trophoblastic invasion of the uterine spiral arterioles [11]. Such alteration in maternal response to trophoblastic invasion leads to placental ischaemia which in turn results in release of certain placental factors which culminate in generalized maternal endothelial cell activation. The generalized endothelial cell dysfunction explains most features of preeclampsia including disturbances in arterial, renal and coagulation mechanisms. These changes in vascular tissue were shown to begin early in pregnancy before the manifestation of clinical symptoms [12].

Obesity is a major risk factor for preeclampsia [13–15]. Preeclampsia risk doubled with each 5–7 kg/m² increase in pre-pregnancy BMI [16]. In obese, non-pregnant individuals, some of the long term cardiovascular morbidities were attributed to obesity mediated systemic inflammation and endothelial dysfunction [17]. Endothelial cell activation and inflammation has been shown to play an integral role in preeclampsia and these have provided intriguing evidences which explain in part, the association of obesity with preeclampsia [18]. Significantly elevated levels of serum interleukin 6 and C-reactive protein as well as evidence of impaired endothelial function were observed in obese pregnant women compared to these with normal body weight [19]. Several clinical and biochemical findings showed that the pathogenesis of preeclampsia are associated with early maternal vascular endothelial cell injury which progressed gradually to the overt disease condition [20]. The erosion and disorganization of the endothelium and its underlying extracellular matrix proteins may therefore contribute to increased release of fibronectin into circulating plasma. Measurement of fibronectin levels in plasma of obese/overweight normotensive women in early pregnancy may therefore serve as additional index of risk and provides possible predictive tools for hypertensive disorders of pregnancy.

Methods

One hundred and sixty three pregnant women attending antenatal care clinic at the University of Calabar Teaching Hospital, Calabar, Nigeria; and sixty non-pregnant control women selected within the same town, participated in the study. One hundred and five pregnant women with normal weight and fifty-eight obese/overweight pregnant women

were grouped into first, second and third trimesters while the sixty control women comprise 40 non-obese and 20 obese/overweight non-pregnant women of child bearing age between 18 and 38 years. Women with chronic hypertension, diabetes mellitus, HIV/AIDS, renal disease and twin pregnancy were excluded from the study.

Information on patient's age and gestational age, as determined by last menstrual period were obtained by oral interview. Patient's status on urine protein and glucose were determined using uristix on spot urine samples. Weight and height were measured with weighing balance and metric rule, respectively.

Body mass index (BMI) was calculated as

$$\text{BMI} = \frac{\text{weight (Kg)}}{[\text{Height (m)}]^2}.$$

Their blood pressures were measured using Accoson sphygmomanometer, whereas, the mean arterial blood pressure (MAP) was calculated as diastolic pressure plus one third of the difference between the systolic and the diastolic blood pressures.

All the participants were provided with verbal information about the study and consent obtained. This work was approved by the University of Calabar Graduate school committee.

Collection and Storage of Sample

About 7 ml of blood was aseptically collected from each participant by venipuncture. Three milliliters aliquot was collected into a pre-cooled plastic tube containing 3.2% buffered citrate in the ratio of 3 ml of blood to 0.3 ml of buffered citrate. The anticoagulated blood was centrifuged at 3,000 rpm for 30 min at 4°C using a refrigerated centrifuge, model MR1822. The plasma was separated and stored in duplicate as aliquots of plasma at a temperature of –20°C. The other 4 ml of the blood was allowed to clot and retract in plain glass tubes at room temperature and the sera were removed into sample tubes after centrifugation at 3,000 rpm for 5 min. Aliquots of serum were also stored frozen at –20°C until required for analysis.

About 77 (47.24%) of the pregnant women were monitored by blood pressure measurement and urine analysis, as they attended the antenatal clinic. This was done till the 40th week of gestation to ascertain whether or not they develop preeclampsia in the course of the pregnancy. Preeclampsia was defined according to World Health Organization (WHO) criteria as blood pressure equal to or greater than 140/90 mmHg (measured at rest in two or more occasions) in the second half of pregnancy and accompanied with proteinuria. Oedema may or may not be present [12].

Biochemical Analysis

Fibronectin levels in the plasma samples were estimated using fibronectin antigen ELISA reagent kit obtained from Technoclone, Wien, Austria. It is a solid phase enzyme immunoassay using monoclonal antibody labeled with a peroxidase enzyme. Total serum protein was determined by Biuret method using Dialab reagent, urea concentration in serum was determined by urease colorimetric method while creatinine was measured by colorimetric two point kinetic, modified jaffes reaction method using Dialab reagent kits purchased from Dialab Produktion, Wien, Austria.

Statistical Analysis

Data obtained from the various groups of patients were compared using student's *t*-test and a level of confidence of $P < 0.05$ was considered significant. Predictive values for the development of preeclampsia were calculated by the method of knottnerus et al. [21].

Results

The result of this study as presented in the Table 1 shows that although mean arterial blood pressures of obese/overweight non-pregnant women were significantly ($P < 0.05$) higher than those with normal body weight, their levels of plasma fibronectin, serum total protein, urea and creatinine were not significantly different. However, fibronectin increased significantly as pregnancy progressed in both the obese and the non-obese group of women (Table 2). The plasma fibronectin and MAP in obese/overweight pregnant women were significantly higher than those with normal body weight in second and third trimesters. Fibronectin in obese/overweight pregnant women also correlated positively with MAP ($r = 0.414$, $P = 0.04$), but there was no correlation in non-obese women (Fig. 1).

The follow up study of 77 pregnant women involving monitoring for subsequent development of preeclampsia revealed that 7(9.09%) of the pregnant women developed preeclampsia before delivery. Plasma fibronectin levels greater than 330 $\mu\text{g/ml}$ (a value above the 95th percentile of normal pregnant women's concentrations) were considered as positive values. Twenty one of the followed up pregnant women (14 of 46 women whose fibronectin levels were measured at 28–32 weeks of gestation and 7 of 31 women whose fibronectins were measured at 18–24 weeks) had positive fibronectin values. On analysis, 28.57% of pregnant women with plasma fibronectin above cut off at 18–24 weeks of gestation developed preeclampsia before delivery and 66.7% of the women who developed

preeclampsia had fibronectin greater than 330 $\mu\text{g/ml}$ at the same gestational age. However, only 14.29% of women with fibronectin greater than 330 $\mu\text{g/ml}$ at 28–32 weeks of gestation developed preeclampsia. These predictive values increased from 28.57 to 40.0% at 18–24 weeks and from 14.29 to 25.0% at 28–32 weeks when only obese/overweight pregnant women were selected for the analysis. When positive fibronectin values and mean arterial blood pressure greater than 90 mmHg were considered together at 18–24 and 28–32 weeks, about 66.7 and 33.3% predictive values were observed, respectively.

Discussion

Fibronectin levels in plasma of non-pregnant women were not significantly higher in obese/overweight women than those with normal body weight (Table 1). Other biochemical parameters were also not different among the two groups. However, the mean arterial blood pressures of obese/overweight women were significantly higher than those with normal body weight. A couple of studies had previously shown evidence of association between high BMI and increased blood pressure [22, 23]. Although, the correlation between obesity and hypertension is well established, the mechanism by which obesity causes hypertension or increased blood pressure is not fully known. Increased level of circulating lipids may be incriminated. Obesity is also known to be associated with insulin resistance and hyperinsulinaemia. It has therefore been speculated that the excess insulin may cause the retention of sodium, expansion of blood volume, production of excess norepinephrine, and smooth muscle cell proliferation most of which are characteristics of hypertension [24, 25].

The concentrations of fibronectin in first trimester pregnant women were significantly lower than those of non-pregnant women. However, the concentrations in second and third trimesters were similar to the non-pregnant level. Fibronectin increased with increasing gestational age in both obese/overweight and non-obese pregnant women (Table 2). The level of fibronectin in obese/overweight pregnant women were significantly ($P < 0.05$) higher than those of their non-obese pregnant counterpart in second and third trimesters. The finding that fibronectin concentration increased as pregnancy progresses agrees with earlier reports for pregnant women with normal body weight in Sweden [26] and Mexico [27]. Panevia-Masin et al. [28], however, found no significant difference in Macedonian pregnant women at 18th, 23rd, 32nd and 36th week of gestation.

The synthesis and release of fibronectin into body fluid has been associated with normal growth of extravillous trophoblasts and the placenta [29, 30]. These sources of

Table 1 Physical and biochemical parameters of obese/overweight and normal non-pregnant women

Parameter	Normal body weight (<i>n</i> = 40)	Obese/overweight (<i>n</i> = 20)
BMI (kg/m ²)	24.36 ± 2.01 (20.5–26.6)	30.41 ± 1.56** (28.7–33.8)
MAP (mmHg)	84.2 ± 6.7 (73.3–96.7)	89.0 ± 1.56** (83.3–100.0)
Total serum protein (g/l)	76.5 ± 6.8 (63.8–94.6)	77.0 ± 5.9 (63.9–86.0)
Fibronectin (μg/ml)	219.30 ± 45.06 (142.0–288.6)	236.60 ± 64.57 (139.0–340.0)
Urea (mmol/l)	3.04 ± 0.64 (2.2–4.4)	2.87 ± 0.49 (2.3–3.8)
Creatinine (μmol/l)	74.33 ± 10.23 (63.6–118.3)	72.30 ± 11.27 (66.3–121.3)

Result are expressed as mean ± SD; the range of data are indicated in brackets

BMI body mass index, *MAP* mean arterial blood pressure, *TSP* total serum protein

** Significant difference at *P* < 0.01 when compared with those of normal body weight

Table 2 Physical and biochemical parameters of obese/overweight and normal pregnant women

Parameter	First trimester		Second trimester		Third trimester	
	Normal <i>n</i> = 25	Obese/overweight <i>n</i> = 8	Normal <i>n</i> = 40	Obese/overweight <i>n</i> = 25	Normal <i>n</i> = 40	Obese/overweight <i>n</i> = 25
BMI (kg/m ²)	24.1 ± 2.3 (20.3–26.6)	30.1 ± 1.23** (28.2–33.4)	25.3 ± 2.4 (21.3–27.0)	32.9 ± 1.7** (30.7–36.2)	26.4 ± 2.9 (22.0–26.8)	33.2 ± 1.82** (31.1–37.7)
MAP (mmHg)	84.1 ± 8.1 (73.3–96.7)	88.3 ± 5.2 (76.7–100.0)	80.9 ± 7.0 (70.0–96.7)	86.3 ± 9.4* (73.3–100.0)	82.8 ± 7.7 (70.0–96.7)	91.1 ± 6.5** (80.0–103.3)
TSP (g/l)	71.8 ± 7.0 (60.1–93.4)	69.8 ± 5.1 (57.3–88.2)	73.9 ± 9.2 (60.4–99.5)	71.9 ± 9.2 (58.2–92.5)	72.3 ± 6.6 (56.8–85.9)	73.0 ± 11.3 (48.3–88.4)
Fibronectin (μg/ml)	166.56 ± 53.3 (98.6–302.9)	189.0 ± 42.21 (121.6–328.4)	197.53 ± 87.3 (86.8–352.9)	270.3 ± 101.6* (110.0–418.0)	235.1 ± 82.7 (114.3–387.6)	305.6 ± 98.2* (158.6–457.3)
Urea (mmol/l)	2.59 ± 0.74 (1.9–4.8)	2.40 ± 0.52 (1.7–3.8)	2.65 ± 0.64 (1.8–4.7)	2.39 ± 0.45 (1.9–3.3)	2.3 ± 0.53 (1.6–3.7)	2.43 ± 0.55 (1.9–4.8)
Creatinine (μmol/l)	59.66 ± 14.0 (44.1–93.7)	66.04 ± 17.6 (43.6–128.5)	67.20 ± 23.3 (40.2–118.3)	83.26 ± 35.9* (46.9–149.7)	62.94 ± 18.2 (42.3–122.1)	93.1 ± 32.3* (49.2–173.4)
Gestational age (weeks)	11.21 ± 3.2 (9.0–14.0)	10.5 ± 2.1 (8.0–13.0)	21.6 ± 2.6 (18.0–25.0)	22.2 ± 2.0 (18.0–24.0)	29.6 ± 2.3 (26.0–37.0)	30.56 ± 3.2 (26.0–36.5)

Result are expressed as mean ± SD; the range of data are indicated in brackets

BMI body mass index, *MAP* mean arterial blood pressure, *TSP* total serum protein

Significant difference at * *P* < 0.05; ** *P* < 0.01 when compared with those of normal body weight in the respective trimesters

Fig. 1 Correlation graph between fibronectin and mean arterial blood pressure in obese/overweight pregnant women. *MAP* mean arterial pressure; *FN* fibronectin

fibronectin may contribute to its increasing level in maternal blood as normal pregnancy progresses. The reason for higher fibronectin concentration in obese/

overweight pregnant women than those of non-obese pregnant women is not well known, but may be related to some level of placental and vascular inflammatory processes attributable to obesity or overweight [31]. Endothelial cells of blood vessels have been known to synthesize cellular fibronectin on activation and release of the protein into the extracellular space at the site of vascular injury is possible [27]. Significant positive correlation (*r* = 0.414, *p* = 0.04) between fibronectin and blood pressure in obese/overweight pregnant women was observed, but none was observed in the pregnant women with normal body weight. These observations may further support the association of increased fibronectin with obesity-associated morbidity. Increased blood pressure as observed in the obese/overweight women may probably result in reduced placental perfusion and increased vascular damage, and could therefore increase the synthesis and release of fibronectin

and proinflammatory products in the obese pregnant women. Elevated levels of plasma fibronectin have been reported in other inflammatory conditions [2].

Significant increase in serum creatinine was also observed in obese/overweight pregnant women compared to women with normal body weight in second and third trimesters. However, there was no difference in first trimesters. Elevated creatinine in obese/overweight pregnant women may be due to decreased renal clearance following haemodynamic changes associated with obesity or overweight. The increased creatinine corresponds with increased MAP observed in second and third trimesters of obese/overweight pregnant women. Increased vascular resistance which characterizes hypertension may reduce renal blood flow and hence impairs renal creatinine clearance. The urea creatinine ratios of the obese/overweight pregnant women were within reference range and were not different from the non-obese women, therefore ruling out any significant damage to the renal tissue.

The follow up of the pregnant women showed that pregnant women with higher plasma fibronectin values were more at risk of preeclampsia than women with lower values. Patients with MAP greater than 90 mmHg were also at risk. Assessment of fibronectin at 18–24 weeks of gestation gave a 28.57% predictive value for preeclampsia as against 14.22% at 28–32 weeks. This suggests that evaluation of fibronectin for the prediction of the disease is better at 18–24 weeks of gestation (mid pregnancy period). Analysis of fibronectin in obese/overweight group of women resulted in increased predictive value up to 40.0% at 18–24 weeks and 25.00% at 28–32 weeks of gestation. This is suggestive of increased risk of preeclampsia among the obese/overweight women. The predictive value was further increased to 66.70% when increased fibronectin above 330 µg/ml and MAP greater than 90 mmHg were considered as a panel.

We thus conclude that measurement of plasma fibronectin at 18–24 weeks of gestation is better in the prediction of preeclampsia than at 28–32 weeks. Also pregnant women with higher level of plasma fibronectin were more at risk of preeclampsia than women with lower values. The risk became higher when mean arterial blood pressure and maternal BMI were also high and we therefore recommend routine measurement of fibronectin in plasma of pregnant women with a view to identifying women with increased risk of preeclampsia especially among the obese/overweight women.

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