Endothelial Dysfunction and Insulin Resistance as Pathophysiologic Mechanisms in a Rat Model of Preeclampsia

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Abstract: Problem statement: To assess the plasma concentrations and placental gene expression of soluble fms like tyrosine kinase (sFlt-1), Vascular Endothelial Growth Factor (VEGF), visfatin and Tumour Necrosis Factor α (TNFα) in a rat model of preeclampsia, induced by chronic Reduction of Uterine Perfusion Pressure (RUPP) and to investigate the involvement of Insulin Resistance (IR) in the pathophysiology of preeclampsia and the possible relation of visfatin and TNFα to IR in preclampsia. **Approach:** Twenty female Sprague-Dawley rats weighing 220-250 g were divided into either RUPP (n = 10) or Normal Pregnant (NP; n = 10) (control) groups. Plasma levels and placental gene expression of sFlt-1, VEGF, visfatin, TNFα, plasma endothelin (ET-1), glucose, serum insulin, creatinine, HOMA-IR and placental Malondialdehyde (MDA) and total antioxidants were measured. Also, Mean Arterial Pressure (MAP), fetal number and weight were determined. **Results:** In RUPP rats, MAP increased, plasma level and placental gene expression of sFlt-1, visfatin and TNFα increased while those of VEGF decreased. Moreover, plasma ET-1, glucose, insulin, HOMA-IR increased while GFR, fetal weight and number decreased. There is a significant positive correlation between TNFα, ET-1, sFlt-1 and MAP, between plasma visfatin or TNFα levels and both serum insulin and HOMA-IR, between visfatin and TNFα, between TNFα and ET-1 and between placental MDA and either sFlt-1 or ET-1. Furthermore, a negative correlation was reported between VEGF and MAP. **Conclusion:** RUPP increased sFlt-1, TNFα and decreased VEGF resulting in endothelial dysfunction which is manifested by increased MDA and ET-1. This results in altered renal function and hypertension. Moreover, IR may be involved in the pathophysiology of preeclampsia. Visfatin and TNFα, may have a role in IR during preclampsia.

Key words: Angiogenesis, endothelial dysfunction, insulin resistance, oxidative stress, preeclampsia, rat

INTRODUCTION

Preeclampsia is a major obstetric problem and a significant source of maternal and neonatal morbidity and mortality. Although preeclampsia continues to affect about 8% of all pregnancies, the incidence of preeclampsia has seen a 40% increase in recent years (Roberts *et al*., 2003). Although preeclampsia has been well characterized, with many studies indicating that proteinuria, edema, endothelial cell dysfunction and insufficient placentation are all hallmarks of this disorder, the mechanisms underlying the pathogenesis of this dreaded condition remain obscure. The continuing uncertainties regarding the mechanisms underlying the pathogenesis of preeclampsia are at least in part attributable to the difficulties in performing mechanistic studies in pregnant women (Granger *et al*., 2006). Thus, the continued characterization and development of animal models for mechanistic research of preeclampsia remains an important endeavor.

There is an imbalance between proangiogenic and antiangiogenic factors, such as Vascular Endothelial Growth Factor (VEGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) in preeclampsia (Lu *et al*., 2007). This dysregulation of angiogenic factors may be important in the pathogenesis of preeclampsia (Lu *et al*., 2007).

The Insulin Resistance (IR) syndrome is comprised of a cluster of metabolic abnormalities that confer increased risk of diabetes, hypertension and cardiovascular disease (Grinspoon, 2006). Several features of the IR syndrome, such as obesity, hypertension, dyslipidemia, systemic inflammation and

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impaired fibrinolysis, are also associated with preeclampsia. In addition, women with polycystic ovary syndrome or gestational diabetes, two disorders characterized by IR, are at increased risk of preeclampsia. Collectively, these data suggest that IR may contribute to the pathogenesis of preeclampsia (Wolf *et al*., 2002). Convincing evidence has been presented that fat-secreted proteins profoundly influence insulin sensitivity and energy metabolism (Fasshauer and Paschke, 2003). Furthermore, various studies suggest that these adipokines (as TNF α) play an important role in the pathogenesis of preeclampsia (Conrad *et al*., 1998; Alhusseini *et al*., 2010). Visfatin is a novel adipokine that mimics the insulin actions, improves glucose tolerance and might play a role in the development of obesity-associated IR and type 2 diabetes mellitus (Fukuhara *et al*., 2005).

Therefore, the aim of this study was to assess the plasma concentrations and placental gene expression of sFlt-1, VEGF, visfatin and TNFα in a rat model of preeclampsia induced by chronic Reduction of Uterine Perfusion Pressure (RUPP). Moreover, the current study aimed to investigate the involvement of IR in the pathophysiology of preeclampsia and the possible relation of visfatin and TNFα to IR in such disease.

MATERIALS AND METHODS

Animals: Twenty female Sprague-Dawley rats weighing 220-250 g were used in the present study. They were purchased from Vaccine and Immunization Authority (Helwan, Cairo, Egypt) and housed (Animal House, Medical Physiology department, Faculty of Medicine, Mansoura University, Egypt) under controlled conditions (temperature 23±1°C and a 12:12 light/dark cycle). The animals were allowed free access to standard rat chow and tap water. The female rats were mated with male rats (275-300 g) and pregnant females were isolated from the males. On day 14 of gestation, rats were randomly divided into either RUPP (n = 10) or Normal Pregnant (NP; n = 10) (control) groups. All experimental procedures of the present study were approved by the Medical Research Ethics Committee of Mansoura University, Egypt.

RUPP procedure: All rats undergoing surgical procedures were anesthetized with 2% isoflurane. Pregnant rats entering the RUPP group underwent the following clipping procedure at 14 days of gestational age. After a midline incision, the lower abdominal aorta was isolated and a silver clip (0.203 mm ID) was placed around the aorta above the iliac bifurcation. Silver clips (0.100 mm ID) were also placed on branches of

both the right and left ovarian arteries that supply the uterus, whereas NP rats underwent a sham procedure (Gilbert *et al*., 2007).

Sampling: At day 20 of gestation, blood samples were obtained from the rat tail vein under ether anesthesia and divided into two tubes: the first one containing K$_2$EDTA, mixed well, centrifuged at 7000 rpm for 10 min to obtain plasma which was stored at -30°C until assay of endothelin, sFLT-1, VEGF, TNFα, visfatin and glucose. Blood in the second tube was collected without anticoagulant, left for 30 min for coagulation and then centrifuged for 10 min at 4000 rpm to obtain serum which was stored at -20 °C until assay of creatinine and insulin. Moreover, rats were housed in metabolic cages for 24 hours to collect urine samples for measurement of urinary creatinine and protein concentrations.

Conceptus measurements: The uterus was exteriorized, the number of viable and resorbed pups was counted and the pups were weighed. The placentae were collected from each uterine horn, snap frozen in liquid nitrogen and stored at -80°C until biochemical analysis of MDA, antioxidants and performing RT-PCR for gene expression.

Biochemical investigations: Plasma glucose was quantitated by glucose oxidase-peroxidase method (Barham and Trinder, 1972, Alhusseini *et al*., 2010). Serum insulin (Ultra Sensitive Rat Insulin ELISA Kit, Crystal Chem INC, Spain, Cat. No. 90060), plasma sFlt-1 (DuoSet ELISA kit, R&D systems, USA, Cat. No., DY471), VEGF (Quantikine ELISA kit, R&D systems, USA, Cat. No., RRV00), visfatin (ELISA kit, RayBiotech Inc., USA, Cat. No., EIA-VIS-1), TNFα (ELISA kit, Thermo Scientific, USA, Cat. No., ER3TNFA), endothelin (EIA kit, Cayman, USA, Cat. No., 583151) were measured according to manufacturer instructions, employing sandwich ELISA method. Plate reader (Tecan, SunRise Absorbance reader) was used for reading. Creatinine in serum and urine were assayed with colorimetric kits. Glomerular Filtration Rate (GFR) was determined by Creatinine Clearance (CrCl). Urinary protein concentration was determined with a Sigma Protein Determination kit (P5656, Sigma Chemical Co) according to manufacturer instructions.

Placental tissue homogenate preparation: Placentae were perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16 mg mL^{-1} heparin to remove any red blood cells and clots. Then, they were homogenized in 5-10 mL cold buffer. Homogenates were centrifuged at 10000 rpm for 15 min at 4°C and

the supernatant was kept at -80°C until assay of lipid peroxides (Malondialdehyde, MDA) and total antioxidants. MDA was analyzed by measuring the production of Thiobarbituric Acid Reactive Substances (TBARS) using colorimetric kit (Bio-Diagnostics, Egypt, Cat. No., MD 2528). Total antioxidants were determined using colorimetric kit (Bio-Diagnostics, Egypt, Cat. No., MD 2528).

Calculation of insulin sensitivity indexes HOMA-IR index was calculated as an estimate of insulin sensitivity. It was calculated, analogous to the assumptions applied in the development of HOMA-IR in humans (Matthews *et al.*, 1985), using the following equation: HOMA-IR = (FPG X FSI)/2.430 (Cacho *et al.*, 2008; Zareian *et al.*, 2010).

RT-PCR: Total RNA extraction was carried out from rat placenta, after homogenization, using TriFast ™ reagent (PeqLab. Biotechnologie GmbH, Carl-Thiersch St. 2B 91052 Erlongen, Germany, Cat. No. 30-2010) according to the manufacturer's instructions. The remaining DNA was removed by digestion with DNase I (Sigma). Semiquantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed using Ready-to-Go. RT-PCR beads for first cDNA synthesis and PCR reaction provided by Amersham Biosciences, England. Cat. No. 27-9266-01, according to the method of Berchtold (1989).

Gene specific primers used were: Gene specific primers were purchased from Biolegio. BV, PO Box 91, 5600 AB Nijmegen, Netherlands. sFlt-1 (VEGF R1): F-5`-CCAAAGCAATTCCCATGC-3`, R-5`-GTA GCC ACG AGTCAAATAGCG-3` (Graepler *et al.*, 2005), TNF α: F-5`-ACAAGCCTGTAGCCCATGTT-3`, R-5`-AAAGTA GAC CTG C C CAGACT-3` (Netea *et al.*, 1996), VEGF: F-5'-GCA CCC ATG GCA GAA GGA G-3', R-5'-TTC TTG TCT TGC TCT ATC TTT CTT TGG-3' (Li *et al.*, 2005), Visfatin: F-5`-GGGAAAGACCATGAGAAAGA-3`, R-5`-AAGGCC ATTG GTTA CAACAT-3` (Choi *et al.*, 2007), GAPDH (Internal control, house keeping gene): F-5'-AAAGGGTCATCATCTCCGC-3', R-5'-GGATGACCTTGCCCACAG-3' (Graepler *et al.*, 2005).

Thermal cycling reaction was performed using thermal cycler (Minicycler PTC-150) with the following program: Initial denaturation: 94°C for 5 min, 30 cycles of 94°C for 0.5 min denaturation, 60°C for 1 min (sFlt-1), 55°C for 30 sec (TNF α), 55°C for 1 min (VEGF), 51°C for 1 min (Visfatin) and 60°C for 1 min (GAPDH) as primer annealing, 72°C for 1 min extension and Final extension at 72°C for 7 min. The products was subjected to agarose gel electrophoresis using 2% agarose stained with ethidium bromide and visualized via light UV Transilluminator (Model TUV-20, OWI. Scientific, Inc. 800 242-5560) and photographed under fixed conditions (the distance, the light and the zoom) The RT-PCR products size were as follow: 316, 427, 360, 468 and 315 bp for sFlt-1, TNF α, VEGF, Visfatin and GAPDH (internal control-house keeping gene) respectively. The results photos were analyzed with scion image ® release Alpha 4.0.3.2. Software for windows ® which performs bands detection and conversion to peaks. Area under each peak were calculated in square pixels and used for quantification. Gene expression levels were determined by calculating the ratio between the square pixel value of the target gene in relation to the control gene (house keeping gene).

Statistical analysis: data were given as mean ± SD and analyzed using SPSS, version 10. $p < 0.05$ is significant.

RESULTS

While basal Blood Pressure (BP) showed non significant change in both Normal Pregnant (NP) (control) and RUPP rats, final BP, measured at day 19 of gestation, is significantly increased in RUPP group compared with the NP rats ($p < 0.05$) as shown in Table 1. Also, protein excretion in urine increased and creatinine clearance (GFR) decreased significantly in RUPP rats relative to control group ($p < 0.05$) (Table 1).

Fasting plasma glucose, fasting serum insulin, HOMA-IR as well as plasma and gene expression of visfatin (Fig. 4) were increased in RUPP rats compared with NP rats ($p < 0.05$) (Table 1). Moreover, plasma and gene expression of sFlt-1 (Fig. 1) and TNFα (Fig. 2) increased while those of VEGF (Fig. 3) decreased in RUPP rats relative to control NP group ($p < 0.05$) (Table 1).
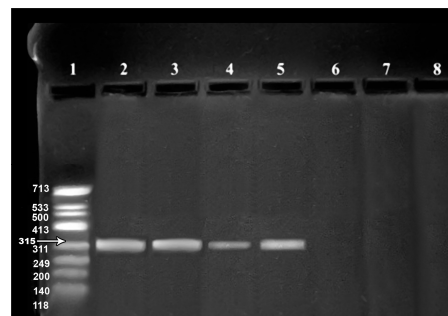

Fig. 1: RT-PCR product of sFlt-1 gene expression: (1) DNA marker; (2) sFlt-1gene in NP rats; (3) sFlt-1 gene of RUPP rats; (4) GAPDH gene of NP rats; (5) GAPDH gene of RUPP rats; (6) negative control

Table 1: Mean Arterial Pressure (MAP), biochemical parameters, placental gene expression and conceptus morphometricus in Reduced Uterine Perfusion Pressure (RUPP) pregnant and Normal Pregnant (NP) rats

Parameters	Normal pregnant rat (control group)	RUPP pregnant rats (preclamptic group)
MAP (mmHg)		
Baseline MAP (mmHg)	107.00±5.00	105.00±5.00
Final MAP (mmHg)	103.00±4.00	129.00±6.00^a
Biochemical parameters		
Urinary proteins (mg/24 h)	15.00±2.00	107.00±9.00^a
GFR (Ccr) (mL min^{-1})	2.21±0.14	1.29±0.22^a
Plasma glucose (mg dL^{-1})	91.50±1.80	125.90±5.70^a
Serum insulin (uU mL^{-1})	8.50±0.70	13.50±0.80^a
HOMA-IR	2.60±0.24	4.90±0.21^a
Plasma visfatin (ng mL^{-1})	0.33±0.02	0.56±0.03$^{a†#}$
Plasma TNFα (pg mL^{-1})	8.10±1.10	19.20±1.50^{a‡†}
Plasma sFlt-1 (pg mL^{-1})	82.40±9.60	665.30±18.60$^{a‡#}$
Plasma free VEGF (pg mL^{-1})	820.40±20.1	410.60±15.80^{a*§}
Plasma endothelin-1 (pg mL^{-1})	1.67±0.20	4.56±0.30$^{a‡#}$
Placental MDA (umol mg^{-1} protein)	1.78±0.21	3.61±0.26$^{a∞σ}$
Total antioxidants (mmol L^{-1})	0.33±0.01	0.16±0.05^a
Placental gene expression		
TNFα gene expression	0.50±0.02	2.60±0.10^a
sFlt-1 gene expression	0.70±0.08	2.80±0.10$^{a#}$
VEGF gene expression	3.20±0.10	0.90±0.07^{a§}
Visfatin gene expression	1.40±0.07	4.90±0.10$^{a#}$
Conceptus morphometrics		
Fetal weight	2.30±0.10	1.90±0.10^a
Number of fetuses	16.00±1.00	6.00±1.00^a

Values are given as mean ± SD for groups of ten rats each. Values are statistically significant at p<0.05. GFR: Glomerular Filtration Rate (creatinine clearance, Ccr); sFlt-1: Soluble fms like tyrosine kinase; VEGF: Vascular Endothelial Growth Factor; MDA: Malondialdehyde; [a]: Significant compared to NP group. Pearson correlation statistical analysis: [†]: Significant positive correlation with serum insulin and with HOMA-IR; [‡]: Significant positive correlation with MAP; [*]: Significant negative correlation with MAP; [#]: Significant positive correlation with TNFα; [§]: Significant negative correlation with sFlt-1; [∞]: Significant positive correlation with sFlt-1; [σ]: Significant positive correlation with endothelin
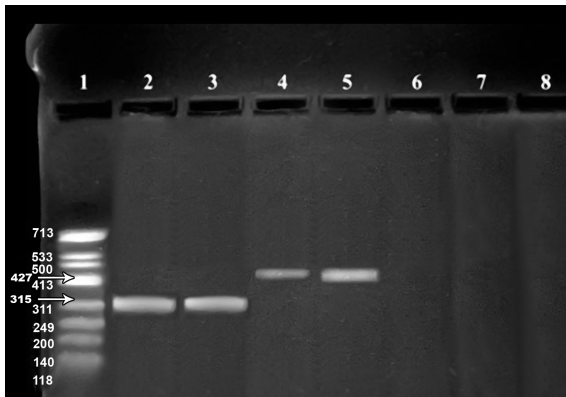

Fig. 2: RT-PCR product of TNF α gene expression: (1) DNA marker; (2) TNF α gene in NP rats; (3) TNF α gene of RUPP rats; (4) GAPDH gene of NP rats; (5) GAPDH gene of RUPP rats (6) negative control

Also, plasma endothlin and placental MDA were increased whereas total antioxidants were decreased significantly in RUPP group compared with control rats (p<0.05) as shown in Table 1.
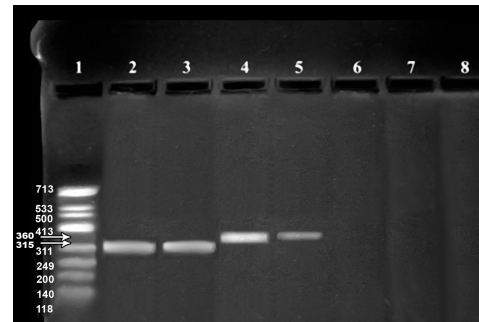

Fig. 3: RT-PCR product of VEGF gene expression: (1) DNA marker; (2) VEGF gene in NP rats; (3) VEGF gene of RUPP rats; (4) GAPDH gene of NP rats; (5) GAPDH gene of RUPP rats; (6): negative control

Table 1 showed that there is a significant positive correlation between TNFα, endothelin or sFlt-1 and MAP (p<0.05) and a negative correlation between VEGF and MAP (p<0.05). Moreover, a significant positive correlation (p<0.05) was reported between plasma visfatin or TNFα level and both serum insulin and HOMA-IR as well as between visfatin and TNFα.
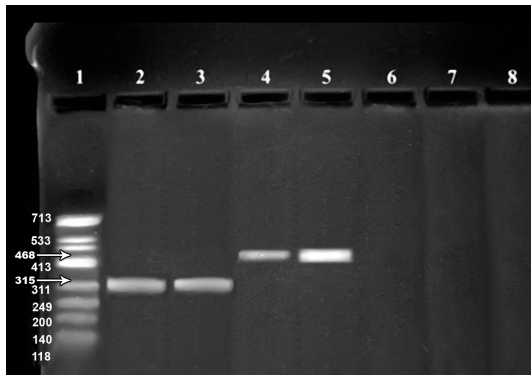
Fig. 4: RT-PCR product of Visfatin gene expression: (1) DNA marker; (2) Visfatin gene in NP rats; (3) Visfatin gene of RUPP rats; (4) GAPDH gene of NP rats; (5) GAPDH gene of RUPP rats; (6) negative control

Also, there is a significant positive correlation between TNFα and endothelin as well as between placental MDA and either sFlt-1 or endothelin (Table 1).

Also, Table 1 showed that the number and weight of RUPP fetuses were significantly lesser than those of NP rats (p<0.05).

DISCUSSION

One of the leading theories concerning the cause of preeclampsia suggests a pathway starting with inadequate trophoblast invasion of maternal spiral arteries leading to decreased placental perfusion. This in turn leads to placental ischemia, resulting in placental release of factors, subsequent maternal endothelial dysfunction and then systemic vasoconstriction (Dekker and Sibai, 1998). Chronic reduction in uterine perfusion pressure in pregnant animals has been used to study potential mechanisms of human preeclampsia because it initiates the disorder at an early step in the cascade described above (Losonczy *et al.*, 1992). The current study showed significant increase in MAP, at the 20th day of pregnancy, decrease in GFR and proteinuria in RUPP rats compared with NP rats (Table 1). These results were consistent with Alexander *et al.* (2001a), who reported that the RUPP model in the rats is characterized by hypertension and urinary protein excretion. Thus, the RUPP model in the rat has many features similar to those of humans with preeclampsia.

The current study showed a significant increase of plasma level and placental gene expression of TNFα in RUPP rats compared with NP rats (Table 1 and Fig. 2). Moreover, there was a positive correlation between TNFα and MAP (Table 1). Placental production rates of several cytokines are altered in preeclampsia. Elevated TNFα expression and secretion by the preeclamptic placenta has been documented (Hung *et al.*, 2004). Moreover, it was reported that serum TNFα increased in RUPP rats and chronic infusion of TNFα into pregnant rats increases arterial pressure and decreases renal plasma flow and GFR (LaMarca *et al.*, 2005a). These findings indicate that TNFα may play a role in mediating the hypertension and reduction in renal hemodynamics observed during RUPP in pregnant rats. TNFα has multiple actions on endothelial cells, including promoting coagulation and inflammatory responses (Granger, 2004) that are particularly relevant in preeclampsia. Within placental tissues, elevated TNFα concentrations may also increased trophoblast apoptosis, leading to accelerated syncytial shedding (a potential trigger of endothelial damage) and impaired placental function (Huppertz and Kingdom, 2004).

The present study revealed increased plasma concentration and placental gene expression of sFlt-1 in the RUPP rats compared with NP rats (Table 1 and Fig. 1). These results were in agreement with Gilbert *et al.* (2007) who reported that maternal plasma and amniotic fluid concentrations of sFlt-1 were increased along with increased levels of immunoreactive sFlt-1 in the placenta of pregnant rats in which RUPP was induced. The present data demonstrated that RUPP is a stimulus for placental production and secretion of sFlt-1.

Moreover, results of this study showed that fetal number and weight were decreased in the RUPP rats relative to normal pregnant control rats (Table 1) which suggest an intrauterine growth restriction. These results were in agreement with previous studies in preeclamptic women (Sibai *et al.*, 2005) and RUPP pregnant rats (Gilbert *et al.*, 2007).

Furthermore, the current study demonstrated that placental gene expression and plasma VEGF are decreased in the RUPP rats compared with NP controls (Table 1 and Fig. 3). Also, there was a negative correlation between plasma and placental gene expression of VEGF and sFlt-1 (Table 1). These results were in agreement with previous studies on RUPP pregnant rats (Gilbert *et al.*, 2007) and humans with preeclampsia (Maynard *et al.*, 2003). It appears from these findings that decreased VEGF is a result of increased sFlt-1. Also, there was a positive correlation between sFlt-1 and MAP and a negative correlation between VEGF and MAP (Table 1), which suggest that increased sFlt-1 and decreased VEGF contributes to hypertension in preeclapmsia. These results were in agreement with Gilbert *et al.* (2007) and Lu *et al.* (2007). Animal studies suggest that antagonism of VEGF activity by sFlt-1 results in hypertension,

proteinuria and glomerular endotheliosis, the classic lesion of preeclampsia (Maynard *et al*., 2003). Soluble Flt-1 in preeclamptic serum has been shown experimentally to inhibit VEGF-mediated angiogenesis; therefore, higher rates of sFlt-1 production and a decline in VEGF activity could be an explanation for the angiogenic abnormalities seen in preeclamptic pregnancies (Ahmad and Ahmed, 2004).

Also, results of this study demonstrated a positive correlation between plasma and placental gene expression of TNFα and sFlt-1 (Table 1). This suggests that increased TNF-α in preeclampsia is associated with elevated sFlt-1 level. Parrish *et al*. (2010) reported that in pregnant rats chronically treated with TNF-α, plasma and placental levels of sFlt-1 increased, therefore they suggest that TNFα may serve as an important stimulus for sFlt-1 production in response to placental ischemia.

The current study demonstrated increased plasma glucose and serum insulin levels and HOMA-IR in rats with preeclampsia (Table 1) which suggests development of IR in RUPP rats compared with NP rats. Increased IR is well established to be associated with preeclampsia (Thadhani *et al*., 2004). Elevated insulin increases sympathetic tone, muscle blood flow and vascular smooth muscle growth (Scherrer and Sartori, 1997).

Moreover, a positive correlation exists between plasma TNFα level and both serum insulin and HOMA-IR (Table 1), which suggest that TNFα may play a role in IR in preeclampsia. Gulati (2005) suggested that the TNF-alpha is involved in preeclampsia and may have a role in gestational diabetes mellitus and IR. TNF-α inhibits insulin receptor signaling and insulin-regulated glucose uptake, suggesting that the IR may be mediated through this cytokine (Kirwan *et al*., 2002).

On the other hand, plasma and placental gene expression of visfatin were significantly increased in RUPP rats compared with NP control rats (Table 1 and Fig. 4). Fasshauer *et al*. (2008) reported that serum visfatin level is elevated in preeclampsia patients. In addition, plasma visfatin was positively correlated with TNF-α (Table 1). Ma *et al*. (2010) reported that the oversecretion of visfatin from placenta, probably induced by the elevated TNF-α level, contributes to the increased serum visfatin concentrations in women with gestational DM. Moreover, visfatin is positively correlated with both serum insulin and HOMA-IR, which suggest its possible involvement in pathogenesis of IR during preeclampsia (Table 1).

The current study also, revealed a significant increase in plasma Endothelin (ET-1) level in RUPP rats compared with NP rats. A positive correlation was reported between endothelin and either MAP or TNF-α

(Table 1). These results indicate the involvement of the vasoconstrictor ET-1 in hypertension during preeclampsia and the association of increased ET-1 with elevated TNF-α in preeclampsia. Since endothelial damage is a known stimulus for ET-1 synthesis, increases in the production of endothelin may participate in the pathophysiology of preeclampsia (August and Lindheimer, 1995). Previously, Alexander *et al*. (2001b) showed that RUPP, in pregnant rats, elicited increased renal cortical and medullary expression of preproendothelin and that chronic administration of the selective Endothelin Type A (ET$_A$) receptor antagonist (ABT-627) markedly attenuates the increased MAP in these rats. In contrast, ET$_A$ receptor blockade had no significant effect on blood pressure in the normal pregnant animal, suggesting that ET-1 plays an important role in mediating the hypertension produced by chronic RUPP pregnant rats (Alexander *et al*., 2001b). Furthermore, a low-dose infusion of TNF-α increased ET-1 mRNA in the kidney, placenta and vasculature (LaMarca *et al*., 2005b). Collectively, these findings suggest that TNF-α -induced hypertension in pregnant rats is mediated in part by endothelin, via ET$_A$ receptor activation.

Oxidative stress has been implicated in the pathophysiology of preeclapmsia because it damages the maternal vascular endothelium. Free radicals released from the poorly perfused fetoplacental unit initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes and converting them to Lipid Peroxides (LPOs) (Chappell *et al*., 1999). The present study showed that placental MDA significantly increased while total antioxidants were decreased in RUPP rats as compared to NP rats (Table 1). Sedeek *et al*. (2008) showed that RUPP in rats caused placental ischemia-induced hypertension which is associated with increased markers of oxidative stress in plasma and placenta (as placental MDA and superoxide production) and decreased plasma total antioxidant status as compared to NP rats. Taken together, these observations suggest that placental ischemia results in generalized oxidative stress in the RUPP model.

Also, results of the present study revealed that there was a positive correlation between placental MDA and either plasma sFlt-1 or endothelin (Table 1). Bridges *et al*. (2009) reported that chronic infusion of sFlt-1 to mimic the increase observed in preeclamptic patients would reduce plasma VEGF concentrations, increase Blood Pressure (BP) and vascular superoxide levels and cause vascular endothelial dysfunction in the pregnant rat. These data indicate that elevated maternal sFlt-1 and decreased VEGF concentrations results in increased oxidative stress which contributes to vascular

dysfunction during pregnancy which in turn may leads to hypertension. Moreover, Fiore *et al.* (2005) demonstrated that ET-1, at concentrations corresponding to values in plasma from preeclamptic women, stimulates the secretion of oxidative stress molecules, such as MDA and simultaneously reduces the production of antioxidant molecules, such as GSH by placental trophoblast cells. Thus endothelin may have additional effects on the maternal cardiovascular system not only by direct actions on the vasculature but also indirectly via oxidative stress. Sedeek *et al.* (2008) showed that a positive feedback loop may exist in which ET-1 both stimulates and is stimulated by oxidative stress.

CONCLUSION

RUPP and placental ischemia may lead to increased sFlt-1, TNFα and decreased VEGF resulting in endothelial dysfunction which is manifested by increased MDA and ET-1, that in turn produce altered renal function and hypertension. Moreover, IR may be involved in the pathophysiology of preeclampsia. Increased visfatin and TNFα, may have a role in IR during preclampsia.

RECOMMENDATION

As IR is a risk factor for preeclampsia, therefore, simple assessments of IR, based on a single determination of fasting insulin and glucose, can predict preeclampsia in pregnant women. Also, controlling IR, obesity and other parameters of metabolic syndrome is recommended to decrease the risk of preeclampsia.

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