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Placental Angiogenic Factors Genes Expression in Preeclampsia: Analysis of Severity Association

[1]Amal A. Mansour, [1]Mai M. Nabil and [2]Ahmed R. Mohamed
[1]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Ain Shams University, Egypt
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, Egypt

Corresponding Author: Amal A. Mansour, Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Ain Shams University, Abbassia, Cairo, 11381, Egypt Tel: +20201111192610

ABSTRACT

The study aimed at the evaluation of angiogenic factors genes, such as Transforming Growth Factor-β3 (TGF-β3) and Urotensin II receptor (UT) genes expression in the placental tissue of women suffering from mild and severe preeclampsia as predictive tests for the progression of preeclampsia. Qualitative and semiquantitative RT-PCR was done for UT receptor and TGF-β3 genes in total 75 placental tissue samples from 50 women with PE (25 with severe PE and 25 with mild PE) and 25 normal pregnant women used as a control group. Both markers increased in the PE group than normo-tensive pregnant ($p \leq 0.01$). There was a significant association between both TGF-β3 and UT positivity and the severe PE ($p \leq 0.01$). The UT and TGF-β3 expression in PE was highly associated with the severity of the PE ($p \leq 0.01$) which suggests them as PE severity biomarkers. The combination between them increased their significance (74% sensitivity with absolute specificity). Women with preeclampsia have high UT levels that are directly related to TGF-β3 and can be considered to play an important role in preeclampsia progression that may have prognostic and therapeutic impacts on these patients.

Key words: Urotensin II receptor, TGF β, multiplex Rt PCR, relative quantitation, preeclampsia

INTRODUCTION

Preeclampsia (PE) is one of the hypertensive disorders that complicate pregnancy after 20 weeks of gestation, characterizes by proteinuria and hypertension and associated with significant morbidity and mortality to both mothers and fetuses (Wang *et al*., 2009).

The precise cause of PE remains elusive but it is believed to be multi-factorial (Grill *et al*., 2009). Multiple risk factors have been described in PE including maternal age, obesity and first pregnancy and pre-existing medical conditions (Hutcheon *et al*., 2011) in addition to the placental hypoxia due to reduced perfusion and endothelial dysfunction and oxidative stress (Mansour and Harb, 2010; Mansour *et al*., 2011a).

Worldwide, the incidence of PE ranges between 2-10% of all pregnancies (Osungbade and Ige, 2011), accounting for 15% of maternal deaths and an estimated 50,000 maternal deaths per year. Preeclampsia may be mild or severe depending on the degree of blood pressure elevation, degree of proteinuria and the presence of signs and symptoms, including epigastric pain, severe headache and blurred vision. However, severe PE can result in bleeding disorders and death (Cross *et al*., 2012). Abnormalities in the angiogenic balance have been proposed to as having a major role in the molecular cascade causing maternal endothelial dysfunction and systemically reduced perfusion in PE (Bdolah *et al*., 2005).

Transforming Growth Factor-β3 (TGF-β3) is one of the angiogenic factors that plays a role in trophoblast differentiation and makes the extravillous trophoblasts to exhibit a less invasive and more proliferative phenotype (Zhao *et al*., 2012; Lee *et al*., 2010).

Urotensin II (UII) is a cyclic peptide that binds to its receptor urotensin II receptor (UT), it elicits an endothelium-dependant vasorelaxation and an endothelium-independent vasoconstriction, UII has also been reported to be a proangiogenic agent increasing angiogenesis both *in vivo* and *in vitro* in endothelial cells (Proulx *et al*., 2008). It is up-regulated in patients with cardiac complications, portal hypertension and other hypertensive disorders, as well as in hypoxic conditions (Albertin *et al*., 2009). High circulating levels of UII have been observed in patients with PE (Balat *et al*., 2005; Gould *et al*., 2010), so it can be a plausible candidate for involvement in the pathogenesis of this disease (Gould *et al*., 2010).

The aim of this study is to assess the expression relationship (UT) and (TGF-β3) genes in placental tissue samples from women with severe and mild PE in comparison with normal pregnant women in order to clarify possible role of these genes in the onset and progression of PE.

MATERIALS AND METHODS

Participants: In a case-control study 75 pregnant women in the 3rd trimester of pregnancy were included in this study. Fifty patients were classified into two groups of PE (ACOG Committee on Obstetric Practice, 2002) mild PE group (25 women mean age: 25.20±5.672, with systolic/diastolic blood pressure equal to or more than 140/90 mm Hg and more than +1 proteinuria on a urine dipstick) severe PE group (25 women mean age: 26.46±6.578, with systolic/diastolic blood pressure equal to or more than 160/110 mm Hg and more than and +2 or more proteinuria on a urine dipstick). In addition to age-matched 25 normotensive healthy pregnant women volunteers (control) their mean age was 25.28±4.596, they shared the same socio-economic status of the PE groups. All subjects were chosen during their routine outpatient checkup at Ain Shams obstetrics hospital clinic, from 2010-2012. All women gave their informed consent to participate in the study which was approved by the Research Ethics Committee of Ain Shams University, Faculty of Medicine. All groups were primigravida and were not under any therapeutic regimen, they didn't have any clinical or laboratory renal insufficiency, chronic hypertension, diabetes mellitus, multiple gestation, neoplasia or neurological disorders.

Tissue samples: Placental tissue samples were obtained immediately after delivery in a Petri dish on ice, tissue blocks (approximately 1 cm^3 each) were dissected from the standard locations on the maternal face of the placentas as previously described (Sood *et al*., 2006). Villous portions were harvested by dissecting free of blood vessels and connective tissue and washing off adherent blood clots by ice cold saline. Villous tissues were wrapped in aluminum foil and immediately stored at -80°C until needed for RNA extraction.

mRNA extraction: The RNA extraction was performed by TriFast™ kit (PeQLab Biotechnologie GmbH Corpo-ration, Erlangen, Germany) which was based on a modified salt precipitation procedure in the presence of highly effective RNase inhibitors and then treated with DNase (Mayer, 1995) and was kept at -80°C till its use in RT-PCR.

Semi-quantitative analysis of UT and TGF-β3 mRNA by multiplex Rt PCR (Mansour *et al*., 2011b): It is a demanding amplification technique which allows the simultaneous detection of several RNA targets in a single tube. Housekeeping β actin with UT and with TGF-β3

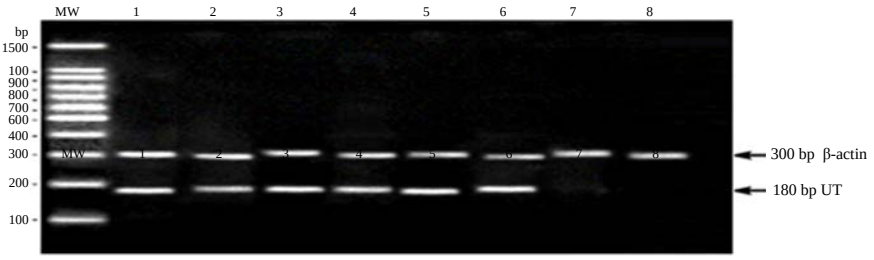

Fig. 1: RT-PCR analysis for placental tissue UT receptors and β-actin RNA (180, 300 bp, respectively) by agarose gel electrophoresis and ethidium bromide staining, MW Lane: Molecular weight 100 bp ladder standard (1500-100 bp), Lanes 1-6: Placental tissue samples from PE patients showing positive UT receptor and β-actin bands, Lane 7 and 8: Two placental tissue samples from the control group show bands of β-actin and no band for UT receptors

primers, respectively were added together in single tubes and multiplex RT-PCR reactions were done for them. Two micrograms of RNA was converted to cDNA using QIAGEN One Step RT-PCR Kit (QIAGEN, USA) by using 3 pm mL^{-1} from each of β actin primers (the sequences of these primers were chosen according to Smith *et al*. (2001) (GenBank accession number 001101.3). Its sense primer was 5'-CTACGTCGCCCTGGACTTCGAGC-3' and antisense primer was 5'-GATGGAGCCGCCGATCCACACGG-3'. Together with 25 pm mL^{-1} of UT sense primer 5′-ACCGTCATGAGCAGCGAGCG-3′ and antisense primer 5′-GGGCAGGCACAGGCTCTTGG-3′(GenBank accession number 018949.1) (Nguyen *et al*., 2012) in a tube and with TGF-β3 sense primer 5′-AGATCTGGGGCGCCTCA-3′ and antisense primer 5′-TGTCGCACGTGGGGTCT-3′ (GenBank accession number 003239.2) (Fahey *et al*., 1996) in another tube. Conditions for RT-PCR were optimized in a Hybaid thermocycler. The experimental procedure was carried out according to the QIAGEN One-Step RT-PCR Kit. First step of Rt was done at 60°C for 60 min, then PCR activation was at 95°C for 15 min. The reaction parameters of UT with the β actin was as follows: Denaturation at 95°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min (for 35 cycles) and then final extension at 72°C for 10 min. The same parameters were used for TGF-β3 with the β-actin tubes except for annealing which was at 60°C for 1 min. The gene products of β-actin, UT and TGF-β3 and were detected at 180, 469 and 300 bp, respectively, as shown in Fig. 1 and 2. All the amplification products were separated on 2% agarose gel and observed by ethidium bromide staining and visualized in a digital imaging system (Molecular Analyst, Bio-Rad, Cambridge, MA).

The semi-quantitation was done by using "Quantity one" computer program version 4.6.3, Bio-Rad Laboratories, USA. Expression of β-actin in each sample was determined as a control for reaction efficiency and to normalize sample to sample variation in RNA amount. The signal intensity in agarose gel of UT and TGF-β3 mRNA for each sample was determined relative to that of β-actin in the same sample. All contaminated wastes included in this study have been sealed and discarded in strong impermeable biohazard bags for further safe transport of theme according to Ain Shams University hospitals infection control biohazard waste disposal policy.

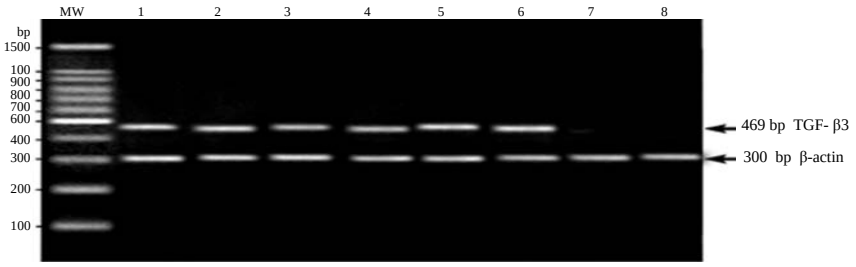

Fig. 2: RT-PCR analysis for placental tissue TGF-β3 and beta actin RNA (469, 300 bp, respectively) by agarose gel electrophoresis and ethidium bromide staining, MW Lane: Molecular weight 100 bp ladder standard (1500-100 bp), Lanes 1-6: Placental tissue samples from PE patients showing positive TGF-β3 and β-actin bands, Lane 7 and 8: Two placental tissue samples from the control group show bands of β-actin and no band for TGF-β3

Statistical analysis: Data were expressed as positivity rates, correlations between variables and the chi-square analysis (χ^2) of the association with category variables. The threshold value for optimal sensitivity and specificity of UT and TGF-β3 were determined by Receiver Operating Characteristics (ROC) curve. The cutoff value that maximized the sum of sensitivity and specificity was chosen for discrimination between normal and PE groups. The specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were calculated according to standard statistical methods. All statistical analysis were performed using the software package SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL). Significant P value considered when it is ≤ 0.05.

RESULTS

Concerning the comparison between severe PE, mild PE and control groups as regards the clinical data and pregnancy outcome, there was a high statistical significant difference ($p \leq 0.01$) between the three groups as regards gestational age, birth weight, systolic and diastolic blood pressure, placental weight and BMI but there was no statistical significant difference ($p > 0.05$) between the two groups as regards maternal age, as shown in Table 1.

The qualitative positivity rate of UT and TGF-β3 is indicated in Table 2. The best cutoff point for UT mRNA to discriminate PE and normal groups using the ROC curve was 0.640 (Fig. 3). The UT mRNA was more than the cutoff value in 80% (20/25) of the severe PE subgroup and in 64% (16/25) of the mild PE subgroup. In the control group no UT expression 0% (0/25), with highly significant difference between the three groups ($p \leq 0.001$). The best cutoff point for TGF-β3 mRNA to discriminate PE and normal groups using the ROC curve was 0.630 (Fig. 3). The TGF-β3 mRNA was more than the cutoff value in 80% (20/25) of the severe PE subgroup and in 48% (12/25) of the mild PE subgroup. In the control group no TGF-β3 expression 0% (0/25), with highly significant difference between the three groups ($p \leq 0.001$), as shown in Table 2. The β-actin was positive in placental tissue samples of all groups of the study.

Expression of UT in placental tissue samples from pre-eclamptic women was detected by RT-PCR which showed 72% sensitivity, 100% specificity, 81.3% accuracy, 100% PPV and 64.1% NPV and for TGF-β3, the values were 64% sensitivity, 100% specificity, 58.1% accuracy, 100% PPV

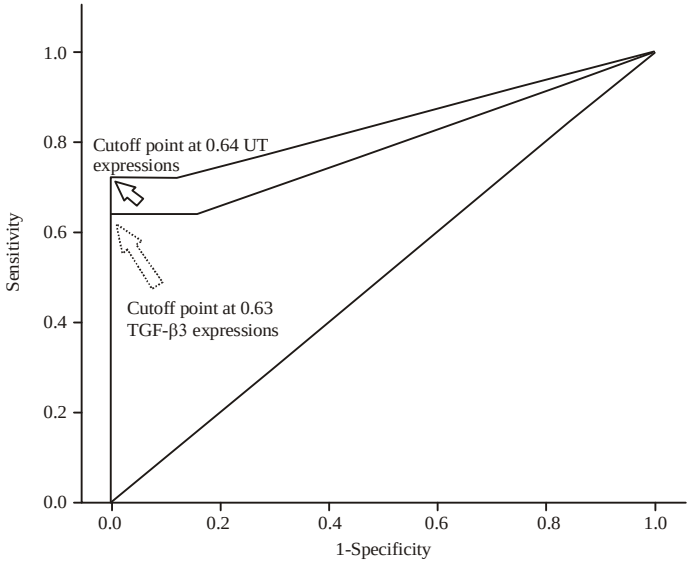

Fig. 3: ROC curve analysis for RT-PCR technique, expression of UT receptor and TGF-β3 gene in severe PE group versus normal group to calculate the best cut off value. For UT receptor II: Arrow denotes cut off point at 0.64 at which UT sensitivity is 72% and specificity is 100% area under the curve is 0.843, standard error is 0.044 and confidence limit is 0.756-0.930. For TGF-β3: Arrow denotes cut off point at 0.630, at which TGF-β3 sensitivity is 64% and specificity, is 100%. Area under the curve is 0.791, standard error is 0.051 and confidence limit is 0.692-0.980

Table 1: Study population demographic and clinical characteristics

Clinical data and pregnancy outcome (Mean±SD)	Severe PE	Mild PE	Control	F	p
Maternal age (year)	26.76±6.578	25.20±5.672	25.28±4.596	0.599	0.552
Systolic blood pressure (mm Hg)	173.60±16.04	134.60±4.89	112.60±8.66	199.7	0.00**
Diastolic blood pressure (mm Hg)	114.00±10.00	92.40±4.36	0.74±5	208.8	0.00**
Gestational age (weeks)	36.12±1.48	38.24±1.87	38.60±1.38	30.1	0.00**
Birth weight (g)	2329.00±410.77	2966.00±279	2978.00±402.61	43.2	0.00**
Placental weight (g)	308.04±13.82	416.88±15	450.00±14.880	651.2	0.00**
BMI	30.24±3.153	27.28±2.836	27.28±4.228	6.1	0.004**

SD: Standard deviation. p value was calculated by one-way ANOVA test. **Highly significant at p≤0.01: PE: Preeclampsia, BMI: Body mass index

Table 2: UT and TGF-β3 positivity rate and semiquantitative RT-PCR analysis in all groups of the study

Semiquantitative RT-PCR	Severe PE (n = 25)		Mild PE (n = 25)		Control (n = 25)		F	p
(UT/β-actin) (Mean±SD)	0.642±0.330		0.510±0.39		0.070±0.188		22.671	0.00**
Range	0.002-0.913		0.002-0.890		0.002-0.590			
Mean rank	50.02		43.14		20.84			
(TGF-β3/β-actin) (Mean±SD)	0.598±0.308		0.370±0.392		0.091±0.207		16.667	0.00**
Range	0.002-0.823		0.002-0.822		0.002-0.510			
Mean rank	50.28		40.28		23.44			
	Severe PE (n = 25)		Mild PE (n = 25)		Control (n = 25)			
Qualitative positivity rate	No.	%	No.	%	No.	%	χ^2	p
UT								
≥0.64	18	72	16	64	0	0	52.321	0.00**
<0.64	7	20	9	36	25	100		
TGF-β3								
≥0.63	16	64	12	48	0	0	20.513	0.00**
<0.63	9	20	13	52	25	100		

**Highly significant at p≤0.01

Table 3: Combined sensitivity, specificity, accuracy, positive predictive value and negative predictive value for UT and TGF-β3 in normal pregnant women versus sever PE

Parameters	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Accuracy (%)
UT	72.0	100	64.1	100	81.3
TGF β3	64.0	100	58.1	100	76.0
Combination of UT and TGF β3	74.0	100	65.8	100	82.7

NPV: Negative predictive value, PPV: Positive predictive value

Table 4: Correlation between placental UT and TGF-β3 expression and pregnancy outcome and clinical data in PE group

	UT receptor		TGF-β3	
Clinical data and pregnancy outcome	Correlation coefficient (r)	p	Correlation coefficient (r)	p
Maternal age	0.156	0.252	0.176	0.220
Gestational age	-0.338	0.017**	-0.414	0.003**
Systolic blood pressure	0.160	0.267	0.178	0.215
Diastolic blood pressure	0.145	0.316	0.201	0.161
Placental weight	-0.182	0.205	-0.326	0.021*
Birth weight	-0.380	0.007**	-0.506	0.000**
BMI	-0.007	0.961	0.021	0.883

Correlation coefficient (r) calculated by pearson's test. *Significant at $p \leq 0.05$ and **Highly significant at $p \leq 0.01$

Table 5: Concordance between expression of both UT and TGF-β3 by semiquantitative RT-PCR in preeclampsia group

		Urotensin II receptor			
TGF-β3	Total	Positive >0.64 n (%)	Negative ≤0.64 n (%)	χ^2	p
Positive >0.63 n (%)	32	31 (86.1%)	1 (7.1%)	27.282	0.00**
Negative ≤0.630 n (%)	18	5 (13.9%)	13 (92.9%)		
Total	100	36	14		

**Highly significant at $p \leq 0.01$, TGF-β3: Transforming growth factor-β3

and 76% NPV but for the combination of both genes, the values were increased to be 74% sensitivity, 100% specificity, 65.8% accuracy, 100% PPV and 82.7% NPV, as shown in Table 3.

About the correlation between placental UT and TGF-β3 expression and pregnancy outcome and clinical data in patients of PE group, the expression of both genes was negatively correlated with gestational age and birth weight with high significance ($p \leq 0.01$). There was positive correlation between the expression of both genes and maternal age, systolic and diastolic blood pressure but this was not significant ($p > 0.05$). About the correlation with BMI, it was negative with UT expression and positive with TGF-β3 expression with no significance in both cases. About the correlation with placental weight, it was negative and not significant with UT but it was negative and significant ($p < 0.05$) with TGF-β3, as shown in Table 4.

High concordance was found between placental expression of both UT and TGF-β3 in PE group, 31 out of 36 cases were positive for expression of both TGF-β3 and UT (86.1%) and 13 out of 14 cases were negative for expression of both TGF-β3 and UT (92.9%) by semiquantitative RT-PCR, as shown in Table 5.

DISCUSSION

Preeclampsia (PE) is a serious hypertensive disorder during pregnancy and remains the leading cause of maternal and neonatal mortalities and morbidities in the world. It is a multi-systemic disease with features such as new onset of hypertension and proteinuria after 20 weeks of gestation. To date, the factors and mechanisms involved in the pathogenesis of PE remains poorly understood (Ma *et al*., 2013).

Urotensin II (UII) is a peptide that binds to its urotensin II receptor (UT) causing vasoconstriction and angiogenesis in endothelial cells. It plays a role in many hypertensive disorders and hypoxic conditions (Zhu *et al*., 2006) and can be a plausible candidate for involvement in the pathogenesis of PE.

The TGF-β family of multifunctional cytokines regulates endothelial cell growth and angiogenesis, affects the nature of immune responses and can affect various functions of trophoblasts. Members of the TGFB superfamily especially TGFβ3 has been found to inhibit the invasion of human trophoblast cells under the hypoxic condition (Chen *et al*., 2010).

In our study, we found that UT mRNA was more than the cutoff value in 72% of the severe PE subgroup and in 64% of the mild PE subgroup. In the control group no UT expression 0%, with highly significant difference between the three groups ($p \leq 0.001$), these results were in consistence with previous study by Gould *et al*. (2010) which quantified differences in UT expression between normal and pre-eclamptic placentas using Western blotting that showed significance ($p \leq 0.05$) this study also hypothesized that increased sensitivity to UII in PE might be achieved by the up-regulation of placental UT and that hypoxia might influence this increase of UT expression via HIF-1*a* (Hypoxia Inducible Factor-1α) over expression.

In our study, we found that TGF-β3 mRNA was more than the cutoff value in 64% of the severe PE subgroup and in 48% of the mild PE subgroup. In the control group no TGF-β3 expression 0%, with highly significant difference between the three groups ($p \leq 0.001$), these results were in consistence with previous studies (Zhao *et al*., 2012; Lee *et al*., 2010) which found increase in TGF-β3 expression in placental tissue of PE.

In this study, there was high concordance between expression of both UT and TGF-β3 by semiquantitative RT-PCR in PE group, samples expressing both genes are representing 86.1% (No. 62) of the total number of samples expressing the two genes (No. 72) and the PE samples not expressing both genes are representing 92.9% (No. 26) of the total number of samples not expressing the two genes (No. 28). According to these findings, we are trying to suggest possible explanations; we are suggesting that placental over expression of HIF-1α due to local hypoxia in PE promotes the expression of both TGF-β3, an inhibitor of extravillous trophoblast differentiation and UT, this considered indicative of persistent hypoxic conditions in the human placenta. Another suggested explanation is that UII can increase the expression of TGF-β1 in cardiac fibroblasts as recorded by Dai *et al*. (2007) and this was mediated by binding to UT, this may occur also between UII and TGF-β3 (Dai *et al*., 2007).

In the present study, there was positive correlation between (maternal age, systolic, diastolic blood pressure and BMI) and expression of both UT and TGF-β3. The UII is a potent vasoconstrictor and TGF-β3 causes exaggerated inflammatory response and generalized maternal endothelial cell activation; subsequently increase the expression of both genes increases systolic and diastolic blood pressure. There is significant negative correlation between (gestational age and birth weight) and expression of both UT and TGF-β3. So we concluded that expression of UT may have a role in pregnancy outcome through its effect on the extent of placental invasion which subsequently affects fetal nutrition, placental weight and birth weight.

As regards the pre-pregnancy BMI in the present study, the mean BMI in kg m^{-2} of severe PE subgroup is 30.24±3.153 kg m^{-2}, while in the mild PE subgroup it is 27.28±2.836 kg m^{-2} and in normal control group it is 27.28±4.228 kg m^{-2}. The difference is significant between the three groups of the study ($p \leq 0.01$) indicating its role in the disease development and progression. This is in consistent with other studies (Na *et al*., 2011; Anderson *et al*., 2012). Na *et al*. (2011) recorded the mean BMI of PE and normal groups were 27.4±4.2 and 26.0±2.9, respectively. Anderson *et al*. (2012) study concluded that obese women require attentive surveillance for the development of PE. The mechanism by which obesity increases the incidence of PE could include increased insulin resistance (McDonald *et al*., 2013). Insulin resistance has been associated with endothelial

dysfunction and increased secretion of endothelin-1, a potent vasoconstrictor. In addition, insulin resistance results in reduction of nitric oxide, increasing the risk of hypertension and cardiovascular diseases (Valerio *et al*., 2011).

CONCLUSION

In conclusion, the results of this study demonstrate that placental expression of UT and TGF-β3 is high in pregnancies complicated with PE in comparison to normal pregnancies that may have a direct role in the pathogenesis of the disease and can be used as therapeutic targets. This can be considered as an attractive point for larger multicentric researches.

REFERENCES

ACOG Committee on Obstetric Practice, 2002. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Int. J. Gynaecol. Obstet., 77: 67-75.

Albertin, G., D. Guidolin, E. Sorato, R. Spinazzi and A. Mascarin *et al*., 2009. Pro-angiogenic activity of urotensin-II on different human vascular endothelial cell populations. Regul. Pept., 157: 64-71.

Anderson, N.H., L.M. McCowan, E.M. Fyfe, E.H.Y. Chan and R.S. Taylor *et al*., 2012. The impact of maternal body mass index on the phenotype of pre-eclampsia: A prospective cohort study. BJOG: Int. J. Obstetrics Gynaecol., 119: 589-595.

Balat, O., F. Aksoy, I. Kutlar, M.G. Ugur, H. Iyikosker, A. Balat and R. Anarat, 2005. Increased plasma levels of Urotensin-II in preeclampsia-eclampsia: A new mediator in pathogenesis? Eur. J. Obstetrics Gynecol. Reprod. Biol., 120: 33-38.

Bdolah, Y., S.A. Karumanchi and B.P. Sachs, 2005. Recent advances in understanding of preeclampsia. Croat Med. J., 46: 728-736.

Chen, Q., L. Chen, B. Liu, C. Vialli, P. Stone, L.M. Ching and L. Chamley, 2010. The role of autocrine TGFβ1 in endothelial cell activation induced by phagocytosis of necrotic trophoblasts: A possible role in the pathogenesis of pre-eclampsia. J. Pathol., 221: 87-95.

Cross, S.N., E. Ratner, T.J. Rutherford, P.E. Schwartz and E.R. Norwitz, 2012. Bevacizumab-mediated interference with VEGF signaling is sufficient to induce a preeclampsia-like syndrome in nonpregnant women. Rev. Obstetrics Gynecol., 5: 2-8.

Dai, H.Y., W.Q. Kang, X. Wang, X.J. Yu and Z.H. Li *et al*., 2007. The involvement of transforming growth factor-β1 secretion in Urotensin II-induced collagen synthesis in neonatal cardiac fibroblasts. Regul. Pept., 140: 88-93.

Fahey, M.S., I.C. Paterson, A. Stone, A.J. Collier and Y.L. Heung *et al*., 1996. Dysregulation of autocrine TGF-β isoform production and ligand responses in human tumour-derived and Ha-ras-transfected keratinocytes and fibroblasts. Br. J. Cancer, 74: 1074-1080.

Gould, P.S., M. Gu, J. Liao, S. Ahmad, M.J. Cudmore, A. Ahmed and M. Vatish, 2010. Upregulation of Urotensin II receptor in preeclampsia causes *in vitro* placental release of soluble vascular endothelial growth factor receptor 1 in hypoxia. Hypertension, 56: 172-178.

Grill, S., C. Rusterholz, R. Zanetti-Dallenbach, S. Tercanli, W. Holzgreve, S. Hahn and O. Lapaire, 2009. Potential markers of preeclampsia: A review. Reprod. Biol. Endocrinol., Vol. 7.

Hutcheon, J.A., S. Lisonkova and K.S. Joseph, 2011. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. Best Pract. Res. Clin. Obstetrics Gynaecol., 5: 391-403.

Lee, S.B., A.P. Wong, K. Kanasaki, Y. Xu and V.K. Shenoy *et al*., 2010. Preeclampsia: 2-methoxyestradiol induces cytotrophoblast invasion and vascular development specifically under hypoxic conditions. Am. J. Pathol., 176: 710-720.

Ma, G., Y. Li, J. Zhang, H. Liu and D. Hou *et al*., 2013. Association between the presence of autoantibodies against adrenoreceptors and severe pre-eclampsia: A pilot study. PLoS One, Vol. 8. 10.1371/journal.pone.0057983

Mansour, A. and H. Harb, 2010. Adiponectin, leptin and oxidative stress in preeclampsia in Egyptian women. Egypt. J. Biochem. Mol. Biol., 28: 31-43.

Mansour, A., H. Harb and M. Abdelhafeez, 2011a. Diagnostic value of homocysteine and other preeclampsia markers: Relationship with severity. Int. J. Biol. Chem., 5: 227-237.

Mansour, A., H. Helmy, M. Ali and S. Kassim, 2011b. Head and neck cancer association to CIAP-2 expression due to human papillomavirus-16 (HPV-16) infection. Egypt. J. Biochem. Mol. Biol., 29: 343-360.

Mayer, M.P., 1995. A new set of useful cloning and expression vectors derived from pBlueScript. Gene, 163: 41-46.

McDonald, S.D., S. Yusuf, M.W. Walsh, E. Lonn and K. Teo *et al*., 2013. Increased cardiovascular risk after pre-eclampsia in women with dysglycaemia. Diabetic Med., 30: e1-e7.

Na, S., J.Y. Shim, B.K. Jung, H.S. Won, P.R. Lee and A. Kim, 2011. Urotensin-II 143 G/A polymorphism is not associated with the risk of preeclampsia in Korean women. Am. J. Reprod. Immunol., 66: 423-427.

Nguyen, T.T., M. Letourneau, D. Chatenet and A. Fournier, 2012. Presence of urotensin-II receptors at the cell nucleus: Specific tissue distribution and hypoxia-induced modulation. Int. J. Biochem. Cell Biol., 44: 639-647.

Osungbade, K.O. and O.K. Ige, 2011. Public health perspectives of preeclampsia in developing countries: Implication for health system strengthening. J. Pregnancy. 10.1155/2011/481095

Proulx, C.D., B.J. Holleran, P. Lavigne, E. Escher, G. Guillemette and R. Leduc, 2008. Biological properties and functional determinants of the urotensin II receptor. Peptides, 29: 691-699.

Smith, S.D., M.A. Wheeler, J. Plescia, J.W. Colberg, R.M. Weiss and D.C. Altieri, 2001. Urine detection of survivin and diagnosis of bladder cancer. J. Am. Med. Assoc., 285: 324-328.

Sood, R., J.L. Zehnder, M.L. Druzin and P.O. Brown, 2006. Gene expression patterns in human placenta. Proc. Natl. Acad. Sci. USA., 103: 5478-5483.

Valerio, E.G., A.L. Muller, S.H. Martins-Costa, J.G.L. Ramos and G. Rodini, 2011. Are insulin resistance index, IGF-1 and metabolic syndrome components correlates with severe preeclampsia? Hypertens. Pregnancy, 30: 302-310.

Wang, A., S. Rana and S.A. Karumanchi, 2009. Preeclampsia: The role of angiogenic factors in its pathogenesis. Physiology, 24: 147-158.

Zhao, H., Y. Jiang, Q. Cao, Y. Hou and C. Wang, 2012. Role of integrin switch and transforming growth factor β 3 in hypoxia-induced invasion inhibition of human extravillous trophoblast cells. Biol. Reprod., 87: 471-477.

Zhu, Y.C., Y.Z. Zhu and P.K. Moore, 2006. The role of Urotensin II in cardiovascular and renal physiology and diseases. Br. J. Pharmacol., 148: 884-901.