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SERUM PREGNANCY-ASSOCIATED PLASMA PROTEIN AND MATERNAL SERUM INSULIN-LIKE GROWTH FACTOR-I LEVELS IN PREECLAMPSIA IN EGYPTIAN WOMEN

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ABSTRACT

Background: Preeclampsia is a severe complication of human pregnancy that occurs in 3–5% of all pregnancies and may threaten maternal and fetal survival. **Objective:** To determine the frequency of the Serum pregnancy-associated plasma protein A and maternal serum insulin-like growth factor-I levels in preeclampsia at second trimester and to verify severity of preeclampsia would be associated with PAPP-A and IGF-I levels. **Methods:** Case-control study included 60women with Preeclampsia, and 30normotensive women as control. Three groups were chosen; preeclampsia group consisted of 30women, mild preeclampsia (MPE) group, 30 women, severe preeclampsia group (SPE) and control group 30 women. All groups were matched strictly for gestational age at second trimester. Maternal blood samples for PAPP-A and IGF-I were collected as soon as the patients at second trimester and compared. The IGF-I was measured with ELISA and The quantitative determination of PAPP-A in the maternal serum was performed with an Immulite / Immulite1000 device using solid phase, chemiluminescence immunometric sandwich method. **Results:** Mean ages of participants, weights and gestational week were similar. Mean PAPP-A and IGF-I levels were significantly lower in preeclampsia groupscompared to control group. However, PAPP-A and IGF-I levels were not different among mild preeclampsia and severe preeclampsia groups. **Conclusion:** PAPP-A and IGF-I levels at

second trimester decrease in all preeclampsia, mild preeclampsia (MPE) group and severe preeclampsia group (SPE), and may be useful in predicting the risk of preeclampsia.

KEYWORDS: PAPP-A, IGF-I, Preeclampsia, Egyptian Women.

INTRODUCTION

Preeclampsia affects approximately 1 in 15 pregnancies[1] and is characterized by a sudden rise in blood pressure and proteinuria which resolves after delivery.[2,3] Currently, there is no effective treatment for preeclampsia other than to induce delivery. However, premature delivery elevates the risk of neonatal death and health problems later in life.[2,4,5] The etiology of preeclampsia thought to involve abnormal placental development.[6,7] It is not a simple complication of pregnancy, but is rather a syndrome of multiple organ failures involving the liver, kidneys and lungs, in addition to coagulation and neural system difficulties. Since cases of severe preeclampsia have a considerably poorer prognosis for both the mother and fetus than uncomplicated pregnancy, it is potentially one of the most devastating pregnancy-associated disorders faced by obstetricians. Pregnancy-associated plasma protein-A (PAPP-A) is a large highly glycosylated protein complex which has been shown to be responsible for the cleavage of insulin-like growth factor (IGF) binding proteins, which are inhibitors of IGF action in several biological fluids. Since, IGFBPs have a key role in modulating IGF activity PAPP-A could be important in regulating fetal growth and development and in trophoblastic invasion of the deciduas.[8] PAPP-A is a well-known first trimester serum marker of pathological pregnancies. Since serum PAPP-A is reduced in the first trimester of pregnancies with fetal trisomies, PAPP-A in combination with other markers has been used for noninvasive early detection of trisomies. It is also reported that maternal serum PAPP-A is reduced in various complicated pregnancies such as fetal growth restriction and preeclampsia.[9]

The insulin-like growth factor (IGF) system comprises the IGF peptides (IGF-I, IGF-II), the cellular IGF receptors (type I, type II), and a family of soluble high affinity IGF binding proteins (IGFBP-1 to IGFBP-6) modulate the bioavailability and activity of the IGFs[10] Insulin-like growth factor-1 (IGF-I) is an important metabolic and mitogenic factor involved in cell growth and differentiation.[11] Most IGF-I is bound to insulin-like growth factor–binding proteins (IGF-BPs) an important regulators of its biological activity.[12] In normal pregnancies, maternal serum levels of IGF-I increase from about the 20th week of pregnancy. This increase is probably induced by placental production of a specific growth hormone (GH)

variant (placental GH), the effects of placental GH on fetal growth are likely to be mediated via circulating maternal IGF-I.[13] In the non-pregnant state, IGFBP-1 produced in the liver and strongly regulated by insulin. In pregnancy, IGFBP-1 also produced in the placenta, resulting in higher maternal concentrations of this protein. It is unknown whether maternal IGFs in early pregnancy are associated with subsequent development of preeclampsia. However, the IGF system is thought to play a role in abnormal placentation, where high decidual production of IGFBP-1 may block IGF-I activity and reduce trophoblastic invasion.[14]

There are inconsistent results regarding the role of IGF-I in the prediction of PE, some researchers reported a lower concentration of IGF-I and a higher concentration of IGFBP-1 in early pregnancy[15] while some studies show higher concentrations of IGF-I and lower concentrations of IGFBP-1 in women with later developed preeclampsia.[16-18] In the present study, we therefore assessed the circulating maternal IGF-I and PAPP-A levels in the second trimester as a predictor for subsequent development of preeclampsia.

METHODOLOGY

Study participants

In the present study, 60 patients with Preeclampsia were collected from the gynecological and Obsteric Department, Mansoura University, Egypt, between August 2015 and September 2016. Three strictly groups were constructed. The first group consisted of 30women who were diagnosed as mild preeclampsia (MPE) during routine prenatal visits at second trimester. The second group consisted of30women who were diagnosed as severe preeclampsia and the third group consisted of30healthy control pregnant whose blood samples were taken during prenatal visits at second trimester of the gestational week that matches the other two groups and these women were followed up to delivery. Ages of women, pregnancy outcomes, blood pressure values, maternal weights and height recorded. Body-mass indexes were calculated. Participants were excluded if they had a pregnancy termination, a major anomaly, a twin pregnancy, or if the pregnancy outcome was unknown, (i.e. if they did not deliver at our hospital). Clinical data and peripheral blood samples were collected only after explaining the objectives of the study and obtaining a signed informed consent form.

Methods

Gestational age was determined from the date of the last menstrual period (LMP) if this differed from the ultrasound estimate by more than 7 days, the ultrasound estimate was used. Blood samples of women in the first three groups were taken as soon as they were diagnosed as preeclampsia. Blood samples of women in the control group were taken at similar gestational ages with the other groups and pregnant women in this group were followed up to term. If any perinatal problems developed such as, labor before 37 weeks or becoming preeclamptic or intrauterine growth restriction or had gestational diabetes, they were excluded from the control group. Preeclampsia was diagnosed when a blood pressure higher than or equal 140/90 mmHg and proteinuria higher than 300 mg/24 h were observed on at least two occasions more than 6 h apart, equal in the presence of any combination of headache, visual disturbances, upper abdominal pain, oliguria(<30 ml/h),hyperbilirubinemia, elevated serum creatinine levels (>0.8 mg/dl), thrombocytopenia(<1,50,000/mm^3) and elevated aspartate or alanine aminotransferase levels. If hemolysis or elevated liver enzymes or a low platelet count was determined, patients were diagnosed as metabolic syndrome. Blood samples of control group were taken from the outpatient pregnancy clinic of our hospital at the mean gestational age matching with other groups and all followed up to delivery. The quantitative determination of PAPP-A in the maternal serum was performed with an Immulite/ Immulite1000 device using solid phase, chemiluminescenceimmunometric sandwich method. Blood samples were centrifuged as soon as they were taken; they were not stored in deep freeze and studied as soon as possible.

The serum samples withdrawn during the second trimester treated according to uniform standards and stored at – 20 0C until analysis. The concentrations of IGF-I was determined by ELISA (DSL-10-2800 Non-Extraction IGF-I ELISA; From Diagnostics Systems Laboratories Inc., Webster, TX, USA) according to the manufacturer's instructions. The IGF-I ELISA has a minimum detection limit of the 2.06 ng/ml (which is the sensitivity according to the DSL kit insert for the non-extraction assay). The intra-assay coefficient of variation (CV) for IGF-I was6.3–8.6 %, the corresponding CVs were 1.7–4.6% as reported by the manufacturer. Samples with concentrations below the detection limits of the respective hormone assays assigned the minimal detection value. The technicians performing the hormone analyses blinded to the clinical outcome.

Statistical analysis

Statistical analysis was performed by SPSS 17.0 package programe. The quantitative data was presented in the form of mean and standard deviation. One-way ANNOVA t-test was used for quantitative data of the studied groups followed by benferroni test to compare between each two groups. Parsons, correlation coeffient was used to study relation between groups. Significance was considered at p value less than 0.05.

RESULTS

Mean gestational ages of the mild preeclampsia, severe preeclampsia and control groups were 30.03± 5.5, 31.83± 3.69and 29.53± 4.36, respectively, and they were similar (group 1 = control group; group 2 = mild preeclampsia; group 3 = severe preeclampsia). Demographic properties and weights were similar (Table 1).

Table. 1: Demographic Characteristics of the Groups.

Parameters	Control (n = 30)	Mild preeclampsia (n = 30)	Severe preeclampsia (n = 30)	p
Age (years)	29.53± 4.36	30.03± 5.5	31.83± 3.69	NS
weight (Kg)	81.1± 12.73	81.4 ± 12.11	86.53 ± 12.98	NS
Gestational age(Weeks)	20.72± 4.66	21.2± 3.57	19.83± 3.93	NS
Body mass index (kg/m2)	25.95 ± 3.56	27.30 ± 4.03	28.57 ± 2.92	NS
Diastolic blood pressure (mmHg)	76.26 ± 3.37	94.33 ± 3.27	105.6 ± 3.23	NS
Systolic blood pressure (mmHg)	114.30± 6.2	149.13± 5.5	167.25± 6.3	NS
PAPP-A (ng/ml)	104± 33.15	80.09± 21.74	75.77± 27.03	0.004*

p value of multiple comparisons with Bonferroni corrections. NS non-significant

* Comparison of group 1 with group 2; ** Comparison of group 1 with group 3 and

*** Comparison of group 2 with group 3

Mean 24 h urinary protein excretion of group 1 was significantly different from group 2 and group 3. Mean urinary protein excretion of group 2 was similar with group 3. Mean PAPP-A levels were significantly lower in preeclampsia compared to their matched control. PAPP-A levels were not different when group with MPE compared with SPE group **(Figure 1).** The concentrations of PAPP-A correlated negatively with maternal Wt, BMI, DBP and SBP (r = - 0.028) (r = - 0.092) (r = - 0.713) (r = - 0.776) respectively. No statistically significant correlation observed between BMI and the concentrations of PAPP-A **(Table 2).**

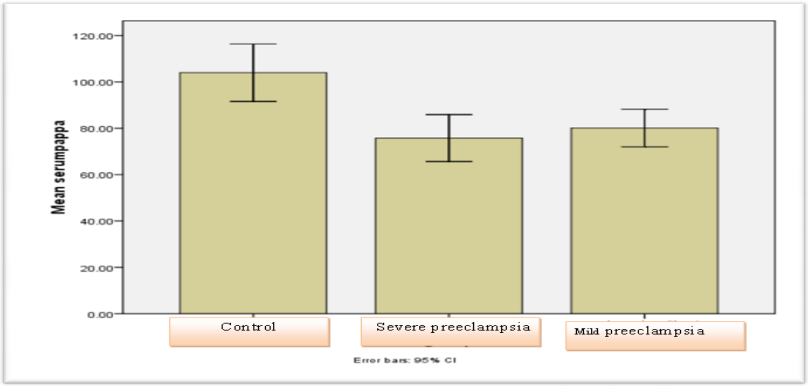

Figure. 1: Serum PAPP-A Level in control and groups with preeclampsia.

Table. 2: Correlation between PAPPA and maternal Wt, BMI, DBP and SBP.

	Wt	BMI	DBP	SBP
Serum PAPPA	- 0.028	- 0.092	- 0.713**	- 0.776**
Sig. (2- tailed)	0.796	0.386	< 0.001	0.001
N	90	90	90	90

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

The maternal characteristics of all groups were presented the age, BMI, and gestational age at sampling **(Table 3 and Figure 1).** The mean level of the serum IGF-I in case of mild preeclampsia (221.1.1+ 114.68) was lower than that in the control group (293.1+ 81.82). It was a statistically significant (P= 0.019). Also, the mean level of the serum IGF-I in case of severe preeclampsia (194.6+ 99.71) was decreased than that in control group and it was found a statistically significant(P= 0.001). On the other hand there was no statistically significant difference in the mean serum IGF-I levels between cases of mild and severe preeclampsia (P= 0.918) **(Table 4 and Figure 4).**

Table. 3: Demographic Data of the studied groups.

	Control (n = 30)	Mild preeclampsia (n = 30)	Severe preeclampsia (n = 30)	p
Age (year)	29.53± 4.36	30.03± 5.5	31.83± 3.69	NS
Weight (Kg)	81.1± 12.73	81.4 ± 12.11	86.53 ± 12.98	NS
Gestational age at sampling(Weeks)	20.72± 4.66	21.2 ± 3.57	19.83± 3.93	NS
Body mass index (kg/m^2)	25.95 ± 3.56	27.30 ± 4.03	28.57 ± 2.92	0.021
Diastolic blood pressure (mmHg)	76.26 ± 3.37	94.33 ± 3.27	105.6 ± 3.23	<0.001
Systolic blood pressure (mmHg)	114.30± 6.2	149.13± 5.5	167.2± 6.3	<0.001

NS, Not statistically significant.

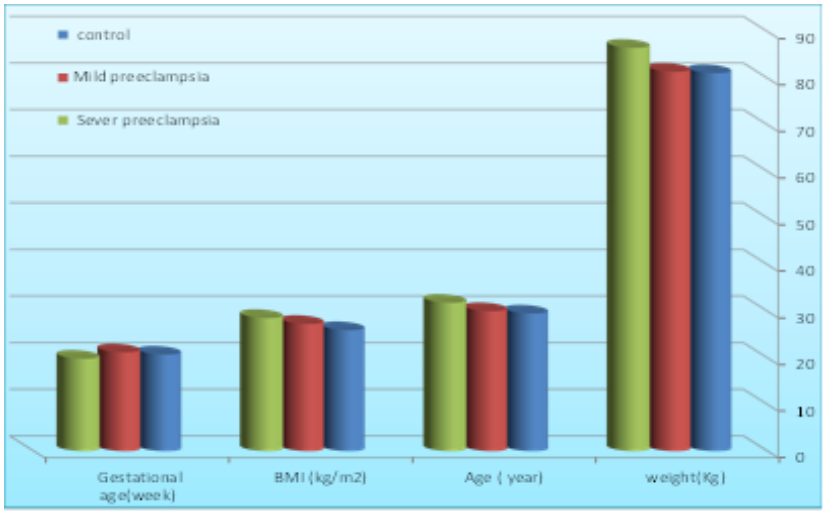

Figure. (2): Bar chart illustrates mean of age, weight, gestational age and MBI among studied groups.

Table. 4: The mean serum levels IGF-1 of the studied groups.

Groups	Mean	SD	
Control	293.1	81.82	P<0.001
Mild precclampsia	221.1	114.68	
Severe precclampsia	194.6	99.71	
Group I versus II			P= 0.019
Group I versus III			P= 0.001
Group II versus III			P= 0.918

The cut off level of serum IGF-1 was 232 ng/ml with high sensitivity 73.3% and high specificity 70% **(Figure 3).** The concentrations of IGF-1 correlated negatively with maternal Wt, BMI, DBP and SBP (r =- 0.028), (r =- 0.214), (r =- 0.376) and (r =-0.335) respectively **(Table 5).**

Table. 5: Correlation between serum IGF-1 and maternal Wt, BMI, DBP and SBP.

	Wt	BMI	DBP	SBP
IGF-1 Pearson Correlation	-0.028	- 0.214-*	- 0.376**	-0.335-**
Sig. (2- tailed)	0.796	0.043	< 0.001	0.001
N	90	90	90	90

**Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

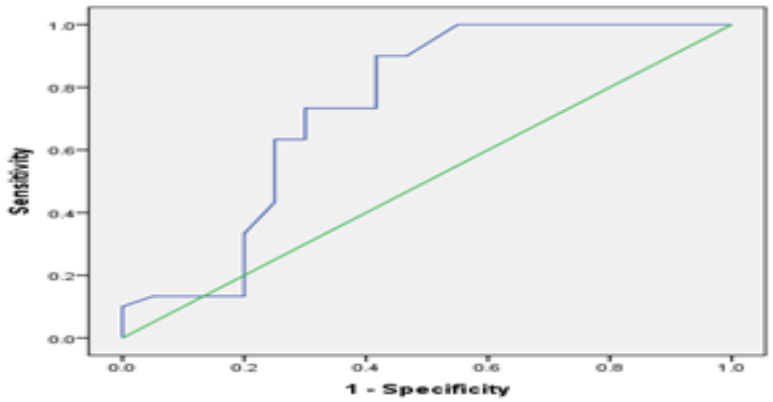

Fig. 3: ROC curve of IGF-1 for control and versus preeclampsia.

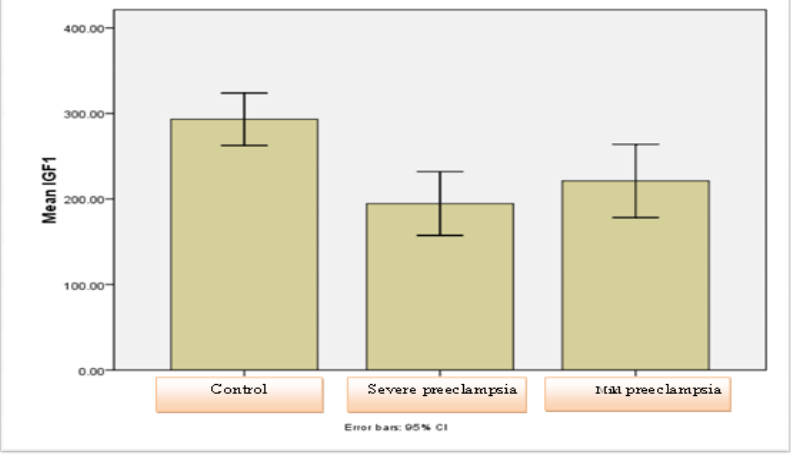

Figure. 4: IGF-1 levels in control and preeclampsia groups.

DISCUSSION

Preeclampsia is a severe complication of human pregnancy that occurs in 3–5% of all pregnancies and may threaten maternal and fetal survival.[19] It is characterized by immunological alterations, systemic inflammation, endothelial dysfunction, and metabolic syndrome.[20,21] Preeclampsia presents with a pattern of typical clinical features (hypertension and proteinuria) and possible further manifestations (renal failure, metabolic syndrome, seizures), but the precise pathogenic mechanisms remain to be determined. Endothelial cell dysfunction is a hallmark of the pathogenesis of the diseased.[22] The clinical syndrome develops from an altered balance between factors produced by the placenta and maternal reaction to them. Immune responses to trophoblast invasion in the early stages (maternal immune adaptation to fetal alloantigens) promote systemic nonspecific inflammation that can lead to the development of the disease later in pregnancy.[20]

Some studies reported that there is no correlation of PAPP-A in preeclampsia but PAPP-A2 instead.[23,24] Uncertainty of specificity of the anti-PAPP-A antibodies possibly underlies this discrepancy. PAPP-A2 was the first protein identified as having a similar structure to PAPP-A.[25] Both PAPP-A and -A2 are involved in the IGF pathway. PAPP-A is a protease that cleaves IGF-binding protein IGFBP4 and IGFBP5, leading to the activation of IGF-II, and I whereas PAPP-A2 has been reported to cleave only IGFBP5.[26,29] Both enzymes expressed in a wide-range of tissues, but abundantly in placental syncytiotrophoblasts and the pregnant uterus. Nishizawa et al.[24] reported that instead of PAPP-A, PAPP-A2 increased in placenta of preeclamptic women compared to uncomplicated pregnancy by western blot and quantitative RT-PCR analysis. They also found that, serum PAPP-A2 levels detected at significantly higher levels in patients with preeclampsia, the pre-eclampsia cut off value of 39.32 ng/l allowed for both high sensitivity (95.0%) and high specificity (85.7%). However, the serum concentrations of PAPP-A did not correlate well with the presence of preeclampsia.[27]

More studies have shown that reduced first trimester serum levels of PAPP-A are associated with subsequent preeclampsia.[28-30] It has been suggested that PAPP-A is useful as a marker of fetal growth restriction at first trimester[31] but there are not many studies concerning late pregnancy levels of PAPP-A at preeclampsia. PAPP-A was first shown to be elevated in the plasma of preeclamptic women nearly 30 years ago. Toop showed elevated levels in severe preeclampsia within 24 h of admission to hospital but PAPP-A values in mild-preeclampsia did not differ significantly from the normal value for the corresponding stage of gestation.[32,33]

Some of the later studies confirmed this postonset elevation of serum PAPP-A.[34-36] Bersinger et al.[34] have measured the concentrations of PAPP-A in placental extracts to specifically study the changes in the placenta in preeclampsia as serum levels reflect cumulative changes in all potential sources. They found that the placental content reflected the serum pattern for PAPP-A in preeclampsia, suggesting that the placenta is a source of the elevated circulating proteins in preeclampsia. Maternal serum levels of PAPP-A have long been known to be depressed in the first trimester of Down's syndrome pregnancies[37], which is widely used in prenatal diagnosis. Other chromosomal abnormalities and adverse pregnancy outcomes, e.g., intrauterine growth retardation and preeclampsia, are also associated with depressed levels of PAPP-A, as is low weight at birth.[38] Our study is

consistent with Bersinger's and Deveci's studies that PAPP-A at the early second trimester (17 weeks) significantly decreases in preeclampsia even in mild form .It was found that, in pregnancies with subsequent preeclampsia PAPP-A, SP1, HPL and PLGF were reduced at 17 weeks of gestation whereas at 25 and 33 weeks only PLGF remained below the controls. In growth-restricted pregnancies PAPP-A, SP1 and HPL were reduced at 17 weeks, and only HPL continued to be strongly affected thereafter.[39] There is also some evidence that serum PAPP-A is reduced in the second trimester in pregnancies that develop PE, but the levels are increased in cases with established disease.[40,41]

The level of blood pressure is only one of the factors representing the severity of preeclampsia. After the cutoff value of 160/110 mmHg, which discriminates the mild and severe-preeclampsia, there is not enough, the finding to conclude that more increase in blood pressure means more severe the disease. That is why, instead of performing correlation analysis between mean blood pressure and PAPP-A levels, we preferred grouping the women as mild preeclampsia and severe preeclampsia according to classical worldwide criteria. We found that PAPP-A at second trimester decreased in both preeclampsia groups and there was not any difference in PAPP-A levels between these two groups representing that PAPP-A level related with severity of the disease but not related with mechanism of preeclampsia. We found that PAPP-A decreased in preeclampsia but level of increment did not differ in separated groups of preeclampsia.

The present study conducted to study the role of the second trimester maternal serum concentration of IGF-I as a prediction of preeclampsia. Our results found the mean maternal serum concentration of IGF-I decreased in both cases of mild and severe preeclampsia, our results were in agreement with that reported by.[42] Some reported that even both early-PE and late-PE are associated with a decrease in maternal serum IGFI that may be evident even late in the first trimester of pregnancy.[43] However, the extent to which this decrease is mediated by different mechanisms depending on the type of PE and the extent to which IGF-I is implicated in the pathogenesis of PE remain to be determined.[44,45] There is extensive evidence that the underlying mechanism for early-PE is impaired trophoblastic invasion of the maternal spiral arteries, reduced placental perfusion, and fetal growth restriction.[46,47] There is evidence that IGF-I is involved in the regulation of trophoblast invasion, placental development and function.[48,49]

On the other hand there was no statistically significant difference in the mean second trimester levels of maternal serum concentration of IGF-I between cases of mild and severe preeclampsia, suggesting that mechanism of development of both types of preeclampsia is a similar mechanism, and genetic mechanisms may influence the severity of preeclampsia.

In contrast, two different research groups reported no difference between IGF-I values in preeclamptic women and normotensive controls, the one referred to preeclamptic women with late-onset disease and the other recruited women with preeclampsia of all subtypes.[50]

It is important to keep in mind that the study was performed in a special group of patients. These findings dictate further mechanistic studies of IGF-I and the level of PAPP-A in the pathophysiology of preeclampsia and indicate confirmatory tests for the predictive value of level of PAPP-A and IGF-I in other populations at different trimesters of pregnancy.

CONCLUSION

The results of this study provide further support to the hypothesis that PAPP-A levels at second trimester pregnancy decrease in preeclampsia. The level of PAPP-A is strongly correlated with severity of the disease. Low PAPP-A is a marker for subsequent development of PE. Also, decreased maternal serum IGFI in the second trimester may be one factor that can be implicated pathogenesis of PE.

REFERENCES

1. Meiri, H., G. Osol, I. Cetin, S. Gizurarson, and B. Huppertz, Personalized Therapy Against Preeclampsia by Replenishing Placental Protein 13 (PP13) Targeted to Patients With Impaired PP13 Molecule or Function. Comput Struct Biotechnol J., 2017; 15: 433-446.
2. von Dadelszen, P., J.M. Menzies, B. Payne, and L.A. Magee, Predicting adverse outcomes in women with severe pre-eclampsia. Semin Perinatol, 2009; 33(3): 152-7.
3. Johnson, M.P., E. Fitzpatrick, T.D. Dyer, J.B. Jowett, S.P. Brennecke, J. Blangero, and E.K. Moses, Identification of two novel quantitative trait loci for pre-eclampsia susceptibility on chromosomes 5q and 13q using a variance components-based linkage approach. Mol Hum Reprod, 2007; 13(1): 61-7.
4. English, F.A., L.C. Kenny, and F.P. McCarthy, Risk factors and effective management of preeclampsia. Integr Blood Press Control, 2015; 8: 7-12.

5. von Dadelszen, P. and L. Magee, What matters in preeclampsia are the associated adverse outcomes: the view from Canada. Curr Opin Obstet Gynecol, 2008; 20(2): 110-5.
6. Gauster, M., G. Moser, K. Orendi, and B. Huppertz, Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. Placenta, 2009; 30 Suppl A: S49-54.
7. Perez-Perez, A., A. Toro, T. Vilarino-Garcia, J. Maymo, P. Guadix, J.L. Duenas, M. Fernandez-Sanchez, C. Varone, and V. Sanchez-Margalet, Leptin action in normal and pathological pregnancies, 2018; 22(2): 716-727.
8. Laursen, L.S., M.T. Overgaard, R. Soe, H.B. Boldt, L. Sottrup-Jensen, L.C. Giudice, C.A. Conover, and C. Oxvig, Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. FEBS Lett, 2001; 504(1-2): 36-40.
9. Sharma N, S.S., Jayashree K, and S.M. Nadhamuni K, Rajagopalan V, Prediction of Intrauterine Growth Restriction in High Pulsatility Index of Uterine Artery. BJMMR, 2017; 22(2): 1-6.
10. Mazerbourg, S. and P. Monget, Insulin-Like Growth Factor Binding Proteins and IGFBP Proteases: A Dynamic System Regulating the Ovarian Folliculogenesis. Front Endocrinol (Lausanne), 2018; 9: 134.
11. Youssef, A., D. Aboalola, and V.K.M. Han, The Roles of Insulin-Like Growth Factors in Mesenchymal Stem Cell Niche. Stem Cells International, 2017; 12.
12. Tian, D., I. Mitchell, and P.K. Kreeger, Quantitative analysis of insulin-like growth factor 2 receptor and insulin-like growth factor binding proteins to identify control mechanisms for insulin-like growth factor 1 receptor phosphorylation. BMC Syst Biol., 2016; 10: 15.
13. Chellakooty, M., K. Vangsgaard, T. Larsen, T. Scheike, J. Falck-Larsen, J. Legarth, A.M. Andersson, K.M. Main, N.E. Skakkebaek, and A. Juul, A longitudinal study of intrauterine growth and the placental growth hormone (GH)-insulin-like growth factor I axis in maternal circulation: association between placental GH and fetal growth. J Clin Endocrinol Metab, 2004; 89(1): 384-91.
14. Vatten, L.J., T.I. Nilsen, A. Juul, S. Jeansson, P.A. Jenum, and A. Eskild, Changes in circulating level of IGF-I and IGF-binding protein-1 from the first to second trimester as predictors of preeclampsia. Eur J Endocrinol, 2008; 158(1): 101-5.
15. Velegrakis, A., M. Sfakiotaki, and S. Sifakis, Human placental growth hormone in normal and abnormal fetal growth. Biomed Rep, 2017; 7(2): 115-122.

16. Grobman, W.A. and R.R. Kazer, Serum insulin, insulin-like growth factor-I, and insulin-like growth factor binding protein-1 in women who develop preeclampsia. Obstet Gynecol, 2001; 97(4): 521-6.
17. Zou, Y., Q. Li, Y. Xu, X. Yu, Q. Zuo, S. Huang, Y. Chu, Z. Jiang, and L. Sun, Promotion of trophoblast invasion by lncRNA MVIH through inducing Jun-B. 2018; 22(2): 1214-1223.
18. Hietala, R., P. Pohja-Nylander, E.M. Rutanen, and T. Laatikainen, Serum insulin-like growth factor binding protein-1 at 16 weeks and subsequent preeclampsia. Obstet Gynecol, 2000; 95(2): 185-9.
19. Harmon, Q.E., L. Huang, D.M. Umbach, K. Klungsoyr, S.M. Engel, P. Magnus, R. Skjaerven, J. Zhang, and A.J. Wilcox, Risk of fetal death with preeclampsia. Obstet Gynecol, 2015; 125(3): 628-35.
20. Redman, C.W. and I.L. Sargent, Immunology of pre-eclampsia. Am J Reprod Immunol, 2010; 63(6): 534-43.
21. Redman, C.W., I.L. Sargent, and A.C. Staff, IFPA Senior Award Lecture: making sense of pre-eclampsia - two placental causes of preeclampsia? Placenta, 2014; 35: S20-5.
22. Peres, G.M., M. Mariana, and E. Cairrao, Pre-Eclampsia and Eclampsia: An Update on the Pharmacological Treatment Applied in Portugal, 2018; 5(1).
23. Yu, M., J. Wang, S. Liu, X. Wang, and Q. Yan, Novel function of pregnancy-associated plasma protein A: promotes endometrium receptivity by up-regulating N-fucosylation. Sci Rep., 2017; 7(1): 5315.
24. Nishizawa, H., K. Pryor-Koishi, M. Suzuki, T. Kato, H. Kogo, T. Sekiya, H. Kurahashi, and Y. Udagawa, Increased levels of pregnancy-associated plasma protein-A2 in the serum of pre-eclamptic patients. Mol Hum Reprod, 2008; 14(10): 595-602.
25. Kjaer-Sorensen, K., D.H. Engholm, H. Kamei, M.G. Morch, A.O. Kristensen, J. Zhou, C.A. Conover, C. Duan, and C. Oxvig, Pregnancy-associated plasma protein A (PAPP-A) modulates the early developmental rate in zebrafish independently of its proteolytic activity. J Biol Chem, 2013; 288(14): 9982-92.
26. Overgaard, M.T., H.B. Boldt, L.S. Laursen, L. Sottrup-Jensen, C.A. Conover, and C. Oxvig, Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase. J Biol Chem, 2001; 276(24): 21849-53.
27. Nishizawa, H., K. Pryor-Koishi, M. Suzuki, T. Kato, H. Kogo, T. Sekiya, H. Kurahashi, and Y. Udagawa, Increased levels of pregnancy-associated plasma protein-A2 in the

serum of pre-eclamptic patients. MHR: Basic science of reproductive medicine, 2008; 14(10): 595-602.

28. Ong, C.Y., A.W. Liao, K. Spencer, S. Munim, and K.H. Nicolaides, First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. Bjog, 2000; 107(10): 1265-70.
29. Yaron, Y., S. Heifetz, Y. Ochshorn, O. Lehavi, and A. Orr-Urtreger, Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn, 2002; 22(9): 778-82.
30. Smith, G.C., E.J. Stenhouse, J.A. Crossley, D.A. Aitken, A.D. Cameron, and J.M. Connor, Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab, 2002; 87(4): 1762-7.
31. Canini, S., F. Prefumo, D. Pastorino, L. Crocetti, C.G. Afflitto, P.L. Venturini, and P. De Biasio, Association between birth weight and first-trimester free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. Fertil Steril, 2008; 89(1): 174-8.
32. Mesdaghi-Nia, E., M. Behrashi, A. Saeidi, M. Abedzadeh Kalahroodi, and M. Sehat, Association between PAPP-A and placental thickness. Int J Reprod Biomed (Yazd), 2016; 14(6): 421-6.
33. Luewan, S., M. Teja-Intr, S. Sirichotiyakul, and T. Tongsong, Low maternal serum pregnancy-associated plasma protein-A as a risk factor of preeclampsia. Singapore Med J., 2018; 59(1): 55-59.
34. Bersinger, N.A., N. Groome, and S. Muttukrishna, Pregnancy-associated and placental proteins in the placental tissue of normal pregnant women and patients with pre-eclampsia at term. Eur J Endocrinol, 2002; 147(6): 785-93.
35. Bersinger, N.A., A.K. Smarason, S. Muttukrishna, N.P. Groome, and C.W. Redman, Women with preeclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. Hypertens Pregnancy, 2003; 22(1): 45-55.
36. Deveci, K., E. Sogut, O. Evliyaoglu, and N. Duras, Pregnancy-associated plasma protein-A and C-reactive protein levels in pre-eclamptic and normotensive pregnant women at third trimester. J Obstet Gynaecol Res., 2009; 35(1): 94-8.

37. Ghoshal, I., B.S. Varashree, V.S. Belle, and K. Prabhu, Correlation of Pregnancy Associated Plasma Protein A and Zinc with Calculated Risk Ratio of Dual Test. J Clin Diagn Res., 2017; 11(9): Bc24-bc26.
38. Temming, L.A., J.M. Dicke, M.J. Stout, R.M. Rampersad, G.A. Macones, M.G. Tuuli, and A.G. Cahill, Early Second-Trimester Fetal Growth Restriction and Adverse Perinatal Outcomes. Obstet Gynecol, 2017; 130(4): 865-869.
39. Bersinger, N.A. and R.A. Odegard, Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. Acta Obstet Gynecol Scand, 2004; 83(1): 37-45.
40. Kirkegaard, I., N. Uldbjerg, and C. Oxvig, Biology of pregnancy-associated plasma protein-A in relation to prenatal diagnostics: an overview. Acta Obstet Gynecol Scand, 2010; 89(9): 1118-25.
41. Atis, A., Y. Aydin, E. Basol, S. Kaleli, F. Turgay, and N. Goker, PAPP-A levels of late pregnancy in preeclampsia and HELLP syndrome. Arch Gynecol Obstet, 2012; 285(1): 45-9.
42. Sun, F., W. Qian, C. Zhang, J.-X. Fan, and H.-F. Huang, Correlation of Maternal Serum Homocysteine in the First Trimester with the Development of Gestational Hypertension and Preeclampsia. Medical Science Monitor : International Medical Journal of Experimental and Clinical Research, 2017; 23: 5396-5401.
43. Anim-Nyame, N., F.A. Hills, S.R. Sooranna, P.J. Steer, and M.R. Johnson, A longitudinal study of maternal plasma insulin-like growth factor binding protein-1 concentrations during normal pregnancy and pregnancies complicated by pre-eclampsia. Human Reproduction, 2000; 15(10): 2215-2219.
44. Conover, C.A., H.B. Boldt, L.K. Bale, K.B. Clifton, J.A. Grell, J.R. Mader, E.J. Mason, and D.R. Powell, Pregnancy-associated plasma protein-A2 (PAPP-A2): tissue expression and biological consequences of gene knockout in mice. Endocrinology, 2011; 152(7): 2837-44.
45. Sifakis, S., R. Akolekar, D. Kappou, N. Mantas, and K.H. Nicolaides, Maternal serum insulin-like growth factor-I at 11-13 weeks in preeclampsia. Prenat Diagn, 2010; 30(11): 1026-31.
46. Yu, C.K., G.C. Smith, A.T. Papageorghiou, A.M. Cacho, and K.H. Nicolaides, An integrated model for the prediction of preeclampsia using maternal factors and uterine artery Doppler velocimetry in unselected low-risk women. Am J Obstet Gynecol, 2005; 193(2): 429-36.

47. Plasencia, W., N. Maiz, S. Bonino, C. Kaihura, and K.H. Nicolaides, Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol, 2007; 30(5): 742-9.
48. Sferruzzi-Perri, A.N., I. Sandovici, M. Constancia, and A.L. Fowden, Placental phenotype and the insulin-like growth factors: resource allocation to fetal growth. J Physiol, 2017; 595(15): 5057-5093.
49. Aplin, J.D., H. Lacey, T. Haigh, C.J. Jones, C.P. Chen, and M. Westwood, Growth factor-extracellular matrix synergy in the control of trophoblast invasion. Biochem Soc Trans, 2000; 28(2): 199-202.
50. Ranke, M.B., Insulin-like growth factor binding-protein-3 (IGFBP-3). Best Pract Res Clin Endocrinol Metab, 2015; 29(5): 701-11.