

Maternal Serum Biochemical Indicators of Trophoblastic Cell and Endothelial Function at First Trimester of Pregnancy

Ranjeeta Gadde¹, Chikkanayakanahalli Doddaiah Dayanand^{1*}, Shimoga Rangappa Sheela²

¹Department of Biochemistry, Sri Devaraj Urs Medical College, SDUAHER, Tamaka, Kolar, India

²Department of Obstetrics and Gynaecology, Sri Devaraj Urs Medical College, SDUAHER, Tamaka, Kolar, India

Email: ranjeetankr@gmail.com, *cd8905@yahoo.co.in, co.ahs@sduu.ac.in

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Abstract

Background: Preeclampsia is a worldwide pregnancy complication, and early identification of patients with an increased risk is one of the key goals in obstetrics. First trimester screening is crucial over the second trimester for understanding the early onset of the disorder, with basal levels of the biochemical parameters associated with the underlying placentation process. **Objective:** The study aims to assess the levels of serum biochemical markers in pregnant women at first trimester, to evaluate statistical significance and correlation of the values in support of trophoblastic cell integrity, endothelial function and oxidative stress. **Materials and Methods:** A longitudinal study was conducted on 86 pregnant women of age group 20 - 35 years, Primigravida with singleton pregnancy who visited prenatal check up between 11 - 13 weeks of gestation. Maternal sera was collected for screening Placental protein 13 (PP13), Caspase 3, Asymmetric dimethylarginine (ADMA), Nitric oxide (NO) by ELISA. Xanthine oxidase (XO) activity was assayed spectrophotometrically. Calcium and Uric acid (UA) were measured by dry chemistry analyser. **Results:** The mean \pm SD values for mean arterial pressure (MAP) are 108.4 ± 18.9 , UA 2.01 ± 0.85 , Total oxidant status (TOS) 12.83 ± 5.17 , Total antioxidant capacity (TAC) 24.10 ± 14.28 , XO 1.01 ± 2.67 , Caspase-3 1.76 ± 2.22 , PP13 489.77 ± 53.6 , Calcium 10.88 ± 1.97 , ADMA 19.03 ± 17.08 and NO 1.16 ± 0.75 . The statistical analysis by SPSS package version 20 revealed positive correlation between ADMA & Caspase-3 ($r = +0.435$), PP13 & NO ($r = +0.241$), TOS & TAC ($r = +0.176$), UA & ADMA ($r = +0.176$), UA & TAC ($r = +0.168$) and negative correlation between PP13 & ADMA ($r = -0.158$), NO & TOS ($r = -0.114$), UA & XO ($r = -0.173$), UA & NO ($r = -0.186$), UA & Caspase 3 ($r = -0.106$) and MAP & Calcium ($r = -0.303$). **Conclusion:** The study concludes that first trimester biochemical markers

and their correlation predict the trophoblastic cell integrity and endothelial function during placentation under prevailing oxidative stress conditions, which may help in identifying women who subsequently go on to develop preeclampsia.

Keywords

Xanthine Oxidase, Placental Protein 13, Caspase 3, Asymmetric Dimethyl Arginine

1. Introduction

Preeclampsia is a pregnancy specific disorder characterized by new onset of hypertension after twenty weeks of gestation with either proteinuria or with thrombocytopenia, renal insufficiency, and impaired liver function pulmonary oedema and cerebral or visual symptoms [1]. The exact aetiology, understanding the underpinning mechanism, prediction, and prevention of preeclampsia is limited and unclear. The precise origin of preeclampsia remains elusive but the impairment in the early placentation is thought to be associated with the pathogenesis of the disorder. Hence, it is one of the leading causes of maternal and neonatal morbidity and mortality affecting 2% - 8% pregnancies worldwide [2]. Till today, there is no therapeutic advancement in the management of preeclampsia and the only treatment is the delivery of the foetus and removal of placenta. So, it is essential to explore potential relevant biochemical markers for its early diagnosis which would allow for timely initiation of preventive therapy. The placenta is known to be a biochemical machinery which is capable of metabolising, synthesizing and secreting a wide variety of molecules which play a key role in early placentation and vascularisation [3]. Thus, any placental pathology linked to impairment of placental development is an essential link with adverse pregnancy complications [4].

Placental protein 13 (PP13), predominantly synthesized in the syncytiotrophoblast is one of the placental proteins involved in early placentation and by virtue of its conserved carbohydrate recognition domain (CRD) specifically binds to β -galactoside residues on the receptors of the endometrium [5] [6]. In normal pregnancy, serum levels of PP13 gradually increase as pregnancy advances [7]. However, decreased first trimester maternal serum PP13 concentrations can be used in the risk assessment for preeclampsia. Despite advanced research, the exact reason for the decrease in PP13 concentration in first trimester is unknown.

Cysteine-aspartic protease-3 (Caspase 3), a key apoptotic enzyme of trophoblastic cell is thought to play an important role in the pathogenesis of preeclampsia [8] [9] [10].

Asymmetric Dimethylarginine [ADMA], an inhibitor of nitric oxide synthase has low plasma levels and exhibits inverse relationship with NO levels in first

trimester of normal pregnancy [11] [12]. However, many studies have reported the elevated levels of ADMA in the first trimester even before the development of preeclampsia [13] [14] [15].

Nitric oxide (NO) is the main vasodilator of the placenta, critical for the maintenance of fetoplacental circulation, placental angiogenesis, reduction of placental bed vascular resistance, inhibiting platelet adhesion and aggregation in the intervillous space [16], diminished NO synthesis contributes to the pathophysiological changes in preeclampsia [17] [18] [19].

Xanthine oxidase (XO) an enzyme oxidant localised in the placenta, myometrium and fetal membrane adds free radicals into the system during catabolism of purines to elevate oxidative stress [20]. Increased XO activity and reactive oxygen species production may influence placental function in preeclampsia [21].

Low concentrations of uric acid (UA) acts as an antioxidant in the first trimester [22], but in high concentrations UA is an inflammatory marker for preeclampsia. Evaluation of UA concentration at early pregnancy is a better diagnostic, predictive and cost-effective marker that might help to identify a high risk group prior to the onset of the clinical symptoms of preeclampsia [23] [24].

Calcium, an important secondary messenger plays a crucial role in blastocyst implantation and in proper placental development and function [25]. Calcium is also essentially required for the secretion of PP13 by exocytosis, [26], for proper functioning of endothelial nitric oxide synthase [27] and in regulation of blood pressure [28].

Mean arterial blood pressure (MAP) has been documented as a biophysical marker and is known to be a better predictor for screening preeclampsia [29] [30].

Oxidative stress represents serum total oxidant status (TOS) and total antioxidant capacity (TAC). Evaluation of each one independently or in combination serves as biological markers to represent oxidative stress in first trimester of normal pregnancy [31] [32].

Research reports documented PP13's significant role in implantation [33], calcium mobilisation [26], lysophospholipase A activity [34] and maternal T cell apoptosis in immune regulation [35]. However, not much information is available on PP13 and its relationship with oxidative stress, placental integrity and endothelial function/ dysfunction. This research gap is the basis for the current study undertaken in the local population. The study highlights the basal values of biochemical parameters in the first trimester of pregnancy with prevailing oxidative stress and endothelial function for early identification of pregnancies at high risk of preeclampsia.

2. Materials and Method

2.1. Materials

The study was conducted in joint collaboration of Department of Biochemistry

and Department of Obstetrics and Gynaecology in R. L. Jalappa Hospital and Research Centre, Kolar, Karnataka, between August 2017-March 2018. Clinically confirmed pregnant women at first trimester enrolled after obtaining informed consent. Ethical clearance for the study was obtained from Central ethics Committee (SDUAHER/KLR/R & D/47/2017-18).

2.2. Sample Collection

The study designed was a longitudinal study, four millilitres of blood was collected from anti-cubital vein under aseptic conditions using vacutainer from first trimester pregnant women visiting Department of OBG for antenatal check-up. Blood was allowed to clot and centrifuged at 3000 rpm to get clear serum. The obtained serum sample was aliquot and stored at -20°C until analysis.

2.3. Inclusion Criteria

This study includes 86 pregnant women, primigravida with singleton pregnancy visited antenatal check-up in first trimester at 11 - 13 weeks of gestation were in the age group of 20 - 35 years. Information on demographic variables, biochemical and haematological parameters of the current pregnancy were obtained.

2.4. Exclusion Criteria

Pregnant women with history of hypertension, liver disease and renal failure, cardiovascular or any vascular diseases were excluded from the study.

2.5. Methods

The measured blood pressure represented as mean arterial blood pressure by using the formula described by Katz 2004 and adapted in research work of Park HJ 2015 [36] [37].

Serum Caspase 3 levels were quantified by sandwich enzyme immunoassay technique according to the procedure provided in the manual by CUSABIO, USA. The absorbance was measured at 450 nm which was directly proportional to the concentration of Caspase 3 in the serum. The detection range is 0.312 ng/mL - 20 ng/mL and sensitivity is less than 0.078 ng/mL.

Serum ADMA levels were quantitated by sandwich enzyme immunoassay technique according to the procedure provided in the manual by CUSABIO, USA. The absorbance was measured at 450 nm which was directly proportional to the concentration of ADMA in the serum. The detection range is 7.9 ng/mL - 500 ng/mL and sensitivity is less than 1.95 ng/mL.

Serum Nitric oxide was estimated by Colorimetric Assay Kit according to the protocol in the manual (Biovision USA), provides an accurate, convenient measure of total nitrate/nitrite in a simple two step process. The first step converts nitrate to nitrite using nitrate reductase. The second step uses Griess reagents to convert nitrite to deep purple azo compound. The amount of azo chromophore accurately reflects nitric oxide amount in the samples. The detection

limit of the assay is approximately 1nmol nitrite/well or 10 μ M.

Serum XO activity was measured by Colorimetric Assay Kit BiovisionUSA, where XO oxidizes xanthine to hydrogen peroxide which reacts stoichiometrically with OxiRed™ probe to generate colour at 570 nm. The kit detects 1 - 100 mU XO in 100 μ L reaction volume.

PP13 assay employs the competitive inhibition enzyme immunoassay technique which is done by the protocol provided by CUSABIO, USA. A competitive inhibition occurs between PP13 and HRP-conjugated PP13 in the pre-coated antibody specific for PP13. The more amount of PP13 in samples, the less antibody bound by HRP-conjugated PP13. The intensity of the colour is measured at 450 nm. The detection range is 2.5 pg/mL - 1000 pg/mL, sensitivity is less than 1 pg/mL.

Serum TOS was measured using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The absorbance was measured under 450 nm, the detection range is 0.2 pg/mL - 60 pg/mL and sensitivity 0.177 pg/mL. Serum TAC) was measured using ELISA, the absorbance was measured under 450 nm, the detection range is 0.5 U/mL - 1200 U/mL, sensitivity 0.411 U/mL. The serum total calcium was measured by Arsenazo III method and UA by Uricase method using dry chemistry analyser Vitros FS5.1 (Johnson and Johnson, USA).

2.6. Statistical Analysis

The obtained results were subjected for statistical analysis using licensed version of SPSS 20.0 software to find out the level of significance and correlation. Data was expressed as mean and standard deviation. Correlations between the biochemical serum markers was done using Pearson's correlation co-efficient (r) and p value less than 0.05 was considered statistically significant.

3. Result

Eighty Six women at their 8 - 11 weeks of gestation were enrolled in the study. **Table 1** and **Table 2** display the demographic and clinical characteristics of the study group. The mean and standard deviation of the study parameters were shown in **Table 3**. The oxidative stress was represented as TOS (12.83 pg/mL \pm 5.17), total antioxidant capacity as TAC (24.10 U/mL \pm 14.28) and Xanthine oxidase activity (1.01 U/mL \pm 2.67) in the first trimester of pregnancy. The trophoblast cell integrity by PP 13 level (489.77 pg/mL \pm 53.6) and Caspase-3 (1.76 ng/mL \pm 2.22). The endothelial function represented in terms of UA (2.03 mg/dL \pm 0.83), total calcium (10.88 mg/dL \pm 1.97), NO (1.16 nmol/ μ L \pm 0.75) and ADMA (19.03 ng/mL \pm 17.08 ng/mL).

The level of significance and correlation of the study parameters were tabulated **Table 4**. This table depicts a moderate uphill positive linear correlation was observed between Caspase 3 and ADMA levels ($r = 0.435$) and it was significant ($p < 0.05$) at the 0.01 level. Similarly, TAC & TOS ($r = +0.5$) in the first trimester values expressed significant correlation at the 0.01 level (2 tailed). However, a

weak positive linear relationship ($r = +0.24$) was observed between PP13 & NO at with a significance ($p < 0.05$) at the 0.05 level, also non-significant correlation was observed between UA & ADMA levels ($r = +0.176$) and between UA & TAC which was non-significant ($r = +0.168$). However, a weak downhill negative correlation was observed between ADMA & PP13 ($r = -0.158$), NO & TOS ($r = -0.103$), UA & XO ($r = -0.173$), UA & NO ($r = -0.186$), UA & Caspase 3 ($r = -0.106$) and between MAP and Calcium ($r = -0.303$).

Table 1. Demographic variables of the pregnant group at first trimester.

No	Variables	Mean	±SD
1	Number of pregnant women	86	
2	Maternal age (years)	23.60	4.00
3	Weight (kg)	50.30	8.99
4	Systolic blood pressure (mmHg)	108.4	9.52
5	Diastolic blood pressure (mmHg)	72.3	8.75
6	Mean Arterial Pressure (MAP)	108.4	18.98

Table 2. Haematological parameters of the pregnant women in the study group at first trimester.

No	Parameters	Mean	±SD
1	Haemoglobin (gm %)	11.12	1.99
2	Platelets ($10^3/\mu\text{L}$)	289.62	79.42
3	Total Count (mm^3)	9.442	2.03
4	MCV (fl/red cell)	81.55	9.56
5	MCH (pg/cell)	26.71	3.43
6	Bleeding time (minutes)	2.00	0.00
7	Clotting time (minutes)	4.581	1.91

Table 3. Showing mean & standard deviation values of the biochemical parameters of pregnant group at first trimester.

No	Parameters	Mean	±SD
1	Random blood sugar (mg/dL)	98.54	17.73
2	Serum creatinine (mg/dL)	0.66	0.125
3	Blood urea (mg/dL)	23.06	4.10
4	Uric acid (mg/dL)	2.01	0.85
5	Total oxidant status (pg/mL)	12.83	5.17
6	Total antioxidant capacity (U/mL)	24.10	14.28
7	Xanthine oxidase (U/mL)	1.01	2.67
8	CASPASE 3 (ng/mL)	1.76	2.22
9	Placental Protein 13 (pg/mL)	489.77	53.6
10	Calcium (mg/dL)	10.88	1.97
11	ADMA (ng/mL)	19.03	17.08
12	Nitric oxide (nmol/ μL)	1.16	0.75

Table 4. Showing positive and negative correlations between the study parameters.

No	Positive correlation	r value	Negative correlation	r value
1	ADMA and Caspase-3	0.435	PP13 and ADMA	-0.158
2	PP13 and NO	0.241	NO and TOS	-0.114
3	TOS and TAC	0.569	Uric acid and XO	-0.173
4	Uric acid and ADMA	0.176	Uric acid and NO	-0.186
5	Uric acid and TAC	0.168	Uric acid and Caspase 3	-0.106
			MAP and Calcium	-0.303

Blood haematological and biochemical parameters in the first trimester were all within the normal range as shown in **Table 1** and **Table 2**. The clinical parameters of the enrolled pregnant women were tabulated in **Table 3** and correlated using Pearson's correlation with p value less than 0.05 was considered statistically significant as shown in **Table 4**. Graphical representation of positive and negative correlations are shown in **Figure 1** & **Figure 2**.

4. Discussion

Several biochemical parameters measured in the study during first trimester of pregnancy are considered to be involved in early placentation, placental endothelial function and also with trophoblast integrity.

ADMA, an endogenous inhibitor of endothelial nitric oxide synthase was positively correlated with the levels of Caspase-3 in first trimester of pregnancy (r value 0.435; $p < 0.05$). Limited data is available on baseline values of ADMA and Caspase-3 in first trimester of pregnancy *in-vivo*, this unique finding is in line with the *in-vitro* findings of Ye S *et al.* (2017) which indicated that ADMA can induce apoptosis via endoplasmic reticulum stress mediated activation of caspase-3 in endothelial progenitor cells [38], Guo W *et al.* (2014) also reported ADMA can induce glomerular endothelial cells apoptosis via PERK and IRE1 endoplasmic reticulum stress apoptosis pathway [39] and Yuan Q *et al.* (2007) presented about the role of ADMA in homocysteine-induced apoptosis of vascular smooth muscle cells, which is related to elevation of intracellular ROS and JNK/p38 MAPK signalling pathways [40]. Similar findings have been reported by Jiang DJ *et al.* that ADMA induces apoptosis of human umbilical vein endothelial cells via elevation of intracellular ROS stress which involves p38 MAPK/caspase-3-dependent signalling pathway [41].

Study results highlighted the relationship of PP13 and placental endothelial function. The serum NO level positively correlated with PP13 in the first trimester of pregnancy generated a clue about the involvement of PP13 with placental vasodilation ($r = 0.241$, $p < 0.05$). The basis for the above findings was an experimental result in animal model, which emphasised the probable role of PP13 in bringing about arterial vasodilation by activating the endothelial signalling pathway in regulation of utero-placental blood flow [42], hypotensive effects,

fetal growth and venous remodelling in pregnant rats [43] [44]. There is scope to conduct studies to demonstrate PP13 therapeutic potential in lowering blood pressure and for improvement of uteroplacental perfusion in humans.

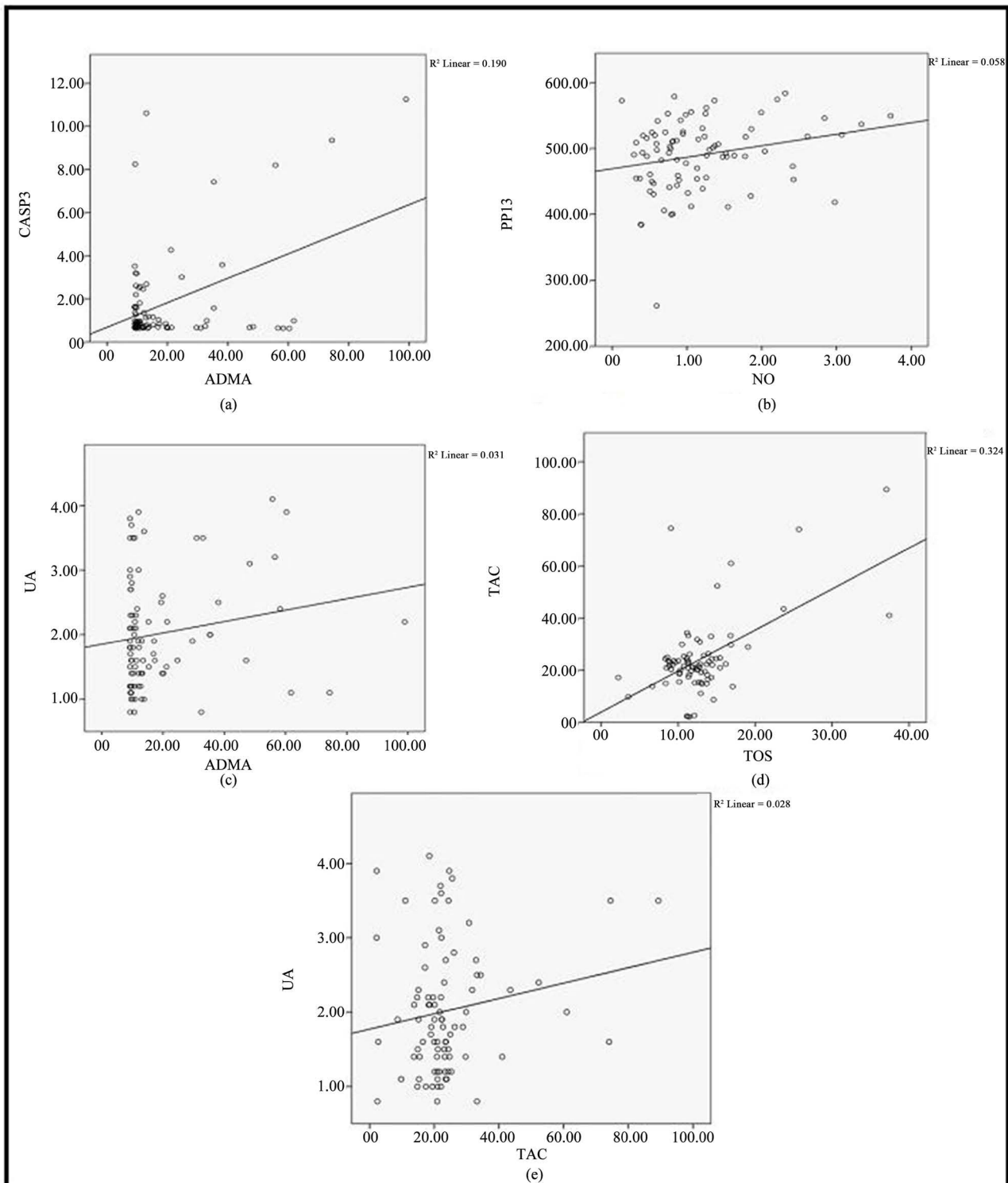


Figure 1. Graphs showing positive correlation between the study parameters. (a) ADMA & Caspase 3 ($r = +0.435$ $p < 0.05$); (b) PP13 & NO ($r = 0.241$ $p < 0.05$); (c) UA & ADMA ($r = 0.176$ $p < 0.05$); (d) TAC & TOS ($r = 0.569$); (e) UA & TAC ($r = 0.168$).

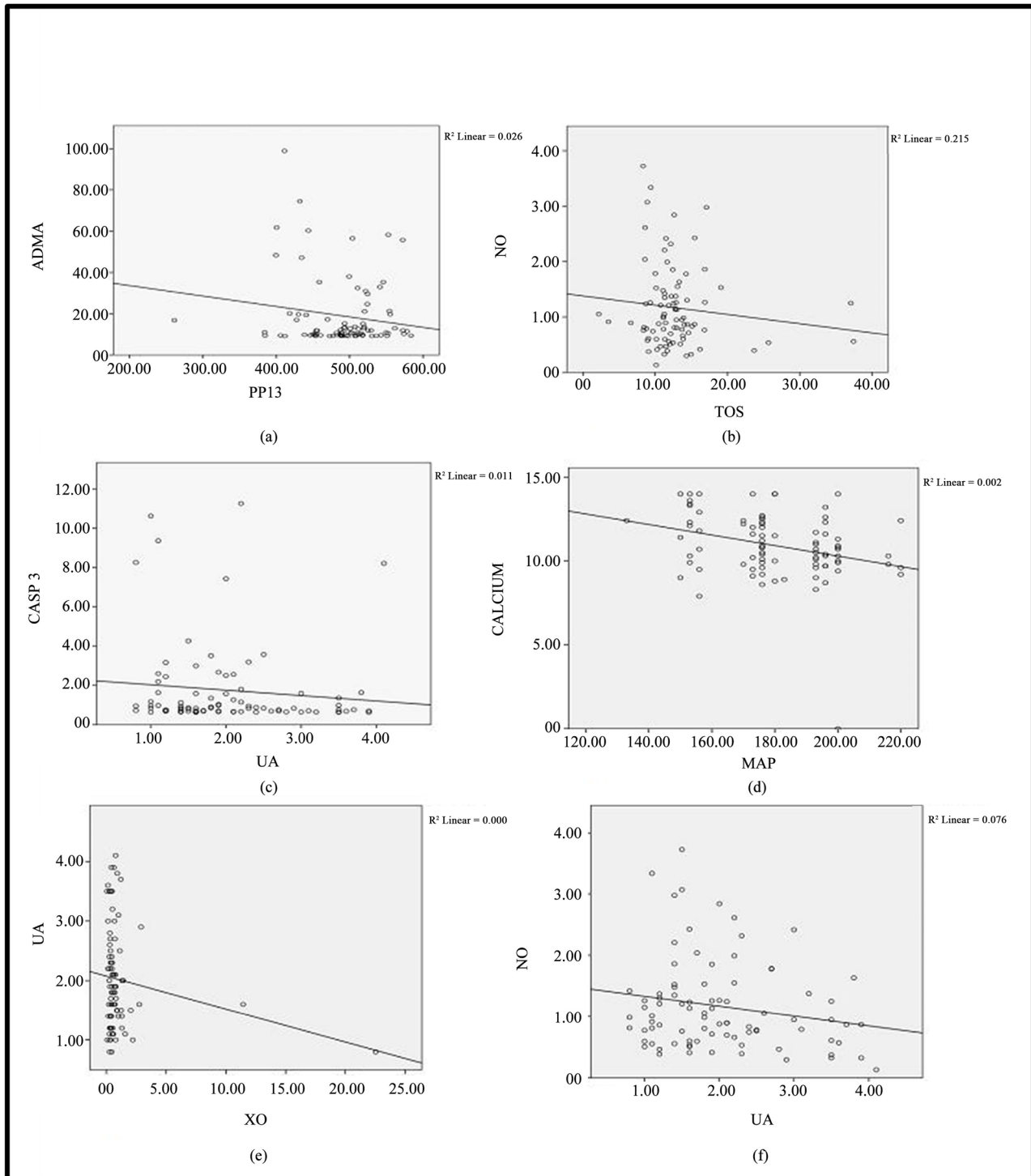


Figure 2. Graphs showing negative correlation between the study parameters. (a) PP13 & ADMA ($r = -0.158$); (b) NO & TOS ($r = -0.114$); (c) UA & Caspase 3 ($r = -0.106$); (d) MAP & Calcium ($r = -0.303$); (e) UA & XO ($r = -0.173$); (f) UA & NO ($r = -0.186$).

Positive correlation was observed between TOS and TAC in the first trimester ($r = 0.569$, $p < 0.05$). It is well documented that oxidative stress prevails during first trimester of pregnancy due to increased reactive oxygen species that influ-

ence a number of functions such as angiogenesis, remodelling of the spiral arteries, cell permeability and vascularisation during embryogenesis and placental development [45]. This oxidative stress has been shown to be compensated by the parallel elevation of antioxidant defences and a defective response in this oxidant stimulus may be an essential component in the aetiology of preeclampsia [46], thus an intricate balance between oxidants and antioxidants is vital in early pregnancy [47].

There are evidences about elevated serum UA [22] [23] and ADMA [48] [49] as pregnancy inflammatory indicators independently. Even though, studies on determination of uric acid, ADMA and its relationship are limited. In the current study, positive correlation between uric acid and ADMA ($r = 0.176$) was observed. Results suggested possible importance of the above parameters as sensitive indicators in understanding gradual onset of preeclampsia.

In the present study, average serum UA quantified in the first trimester of pregnancy is 2.01 mg/dL. At this concentration, UA exhibits efficient antioxidant properties in early pregnancy [22] [46]. Results showed positive correlation between serum UA and TAC ($r = +0.168$), it is well documented in many studies that uric acid is an endogenous aqueous antioxidant, the main contributor of antioxidant capacity of human plasma [50] [51] [52] [53] [54].

Study results showed positive correlation between UA and ADMA with no statistical significance in the first trimester of pregnancy. Both maternal serum UA and ADMA levels are reduced in normal pregnancy but increase as gestational age increases [55] [56]. In early pregnancy, the reduction in UA levels is due to the effects of estrogen, expanded blood volume and increased glomerular filtration rate. Similarly, the reduction in ADMA may lead to hemodynamic adaptation, an increased organ perfusion and uterine relaxation for the proper fetal growth.

There exists paucity of information on correlation between maternal serum PP13 and ADMA levels in first trimester of pregnancy. Our current experimental approach performed to determine whether PP13 from syncytiotrophoblast origin has any influence on ADMA metabolite more precisely involved in regulation of NO production. In the study context, we found negative correlation between maternal serum PP13 & ADMA levels ($r = -0.158$, $p < 0.05$) in the first trimester.

Negative correlation was found between the values of NO & TOS ($r = -0.114$), UA & XO ($r = -0.173$), UA & NO ($r = -0.186$), UA & Caspase 3 ($r = -0.106$) and MAP & Calcium levels ($r = -0.303$) in the first trimester of pregnancy.

5. Conclusion

Study concludes that, mean arterial blood pressure was effectively correlated with calcium, during placentation and measured ADMA, XO, Caspase 3, PP13, NO, TAS, TOS in first trimester, and represents trophoblastic cells integrity and endothelial function in the prevailing oxidative stress conditions. PP13 was posi-

tively correlated with NO and negatively correlated with ADMA in the first trimester, which represents proper endothelial functioning during fetal development. The same bio-molecules values in the second trimester of the same group facilitate to record the possibility of developing pregnancy complications. The study has limitation with respect to control group, second trimester cases and sample size.

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Conflict of Interest

The authors declare that there was no conflict of interest that would prejudice the impartiality of this scientific work.

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