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Study of uterine artery perfusion and total antioxidant capacity in second trimester pregnancy

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Abstract
Background: Abnormal placentation during the 1st trimester results in oxidative stress, affecting the subsequent gestational course. This study evaluated if the condition of pathological perfusion is distinguished by an altered plasma antioxidant capacity along with investigating the efficacy of total antioxidant capacity (TAC) measurement as an adjunct to doppler sonography.
Methodology: Pregnant women (2nd trimester), categorized into 2 groups [Control (n=25, normal uterine perfusion) and case (n=25, abnormal uterine perfusion)], were evaluated for TAC. Statistical significance was set at $p \leq 0.05$.
Results: Mean plasma TAC was higher in the control (750.02±74.12 µmol/l) than the case group (580.97±168.37 µmol/l) ($p<0.0001$). Difference in the mean period of gestation between groups was significant ($p<0.0001$). The gestation period and birth weight in case group was lower than that of control.
Conclusion: Hindered uterine perfusion causing reduced plasma TAC levels was observed. Estimating TAC may have diagnostic, pathophysiologic and therapeutic implications concerning abnormal uterine perfusion.

Keywords: antioxidants, oxidative stress, placentation, pregnancy, uterine anomalies

1. Introduction
A particular physiological change experienced by a woman during her lifespan is the 20-fold pregnancy rise in the uterine artery blood flow [1]. This evaluative factor contributes towards the preservation of the intrauterine environment, thereby permitting normal placental functioning to support the fetal growth and development by delivering the required nutrition, waste removal and most importantly in delivering oxygen to the fetoplacental unit that is directly limited by the uterine blood flow [2].
A defective placentation could result in the inadequate development of uterine vasculature, leading to developing preeclampsia and fetal growth restriction (FGR). The association between uterine arterial blood flow and the developing placenta is vital in understanding normal placentation and its disruption with respect to preeclampsia and FGR [2]. Around 40,000 women, mostly from developing countries, succumb annually due to preeclampsia or eclampsia. Preeclampsia alone is estimated to account for about 40% to 60% of maternal deaths in developing countries [3]. In the Indian scenario, rate of preeclampsia varies from 5-15% and of eclampsia is about 1.5% [4].
Pregnancy is affiliated with several metabolic changes, with higher levels of oxidative stress being a vital factor concerning the pathology of pregnancy related disorders [5]. During pregnancy, a physiological response towards the fetoplacental energy demands is the intrauterine oxidative stress [6]. During the 1st trimester, antioxidant enzyme concentration and activity levels are reduced, which makes the trophoblastic cells vulnerable to oxygen-mediated damage. Abnormal placentation during the 1st trimester results in oxidative stress, which in turn plays a vital role in terms of pregnancy complications [7]. The beginning of 2nd trimester accompanies a rise of maternal arterial flow, due to which the placenta experiences an explosion of oxidative stress, which could affect the placental functioning, thereby modifying the subsequent gestational course [8].
Detecting the probability of developing any such complications at an earlier stage in pregnancy is of paramount importance. Close maternal surveillance and the potential to provide preventive treatment can greatly improve pregnancy outcome and decrease maternal complications. Ideally a screening test with a low false negative rate and a low false positive rate would fulfil this criterion.

To implement such a test in large population, it must be easy, inexpensive, convenient for the mother, quick, simple, easily interpretable, and reproducible. Several tests have been recommended in identifying women who are at a peril of developing preeclampsia. During the 2nd trimester, measuring uterine perfusion by employing the Doppler ultrasound is a feasible, non-invasive method in identifying women at a risk for both intrauterine growth restriction (IUGR) and preeclampsia [9]. Although the sensitivity and predictive values are limited, at present, no other preferred method is available for early risk assessment [10]. However, not all patients with disturbed uterine perfusion develop pregnancy related complications resulting from a failed trophoblast invasion and a persistent high uteroplacental resistance [10]. Thus, additional parameters are required to improve predicting the outcome of an abnormal pregnancy.

Recent studies suggest oxidative stress and altered endothelial cell function may occur because of pregnancy induced hypertension. Increase in generation of free radical (escalating lipid peroxide levels) is reported [11]. But there is inadequate knowledge concerning the antioxidant status with respect to normal and preeclamptic pregnancies and there are only scattered details for healthy pregnant women concerning the baseline antioxidant levels. Therefore, the main focus of the study was to evaluate if the condition of pathological perfusion is distinguished by an altered plasma antioxidant capacity and vice-versa along with investigating the utility of total antioxidant capacity (TAC) measurement as a complementary tool to doppler sonography.

2. Materials and Methods

2.1 Study design

This prospective study was conducted at a tertiary care centre in Bengaluru, Karnataka, India, from November 2011 to April 2013. Approval from the Institutional Ethics Committee (STD-1/EC/0450/2011) and a written consent from the participants were acquired before commencement of the study.

2.2 Study subjects

The study involved 50 singleton pregnant women in their 2nd trimester (22-26 weeks of gestational age), aged between 18-35 years. They were categorized equally into control (normal uterine perfusion) and case (abnormal uterine perfusion) groups. Pregnant women excluded from this study were those with multiple gestation, diabetes mellitus, anemia, history of chronic hypertension or consumption of vitamin A, E and C supplements.

The formula represented below was applied to calculate the sample size:

$$n = \frac{2\left(Z\alpha_{/2} + Z_{1-\beta}\right)^2}{d^2}$$

Where effect size $d = \frac{|\mu_1 - \mu_2|}{\sigma}$ where $\sigma = \sqrt{\frac{(n_1-1)*(s_1{}^2)+(n_2-1)*(s_2{}^2)}{(n_1+n_2-2)}}$

Where μ_1 = mean of the first group, μ_2 = mean of the second group, σ^2 = pooled variance and $s_1{}^2$ and $s_2{}^2$ = standard deviations in the two groups respectively

By assuming large effect size d=0.8 in TAC between the study groups, for 95% level of significance $(Z_{\alpha/2})$ value is 1.96 and for 80% power $(Z_{1-\beta})$ value is 0.84, sample size required was 25 per group.

2.3 Study procedure

Since difference in diet habits between controls and cases was insignificant, simple random sampling technique was employed to select the participants. All participants undergoing ultrasound were routinely offered a color Doppler ultrasound examination to assess uterine perfusion. Patients were later evaluated for TAC by performing the Ferric Reducing Antioxidant Power (FRAP) assay as per Benzie and Strain [12]. Patients were followed up till their delivery to assess the outcome.

2.4 Statistical analysis

Data analysis was performed using the R i386 3.6.3 software. Categorical variables, represented in numbers (%), were compared by performing the Chi-square test. Continuous variables, represented as mean ± standard deviation (SD), were compared by performing the t-test/welch t-test/Mann-Whitney U-test/ANOVA. Simulation was performed when the expected cell count was <5. Level of significance was considered at $p \leq 0.05$.

3. Results

The mean age of participants in this study was 26.48±5.12 years, and majority of them, from both the groups, belonged to 26–30-year age group. Greater number of women were multigravida (control: n=13, 52%; case: n=14, 56%). During the doppler assessment, the mean of systolic blood pressure (SBP) and diastolic blood pressure (DBP) was significantly higher in cases. The intergroup difference in SBP (p<0.0001) and DBP (p=0.0006) was significant (Table 1).

Table 1: Distribution of age, gravida, and blood pressure status of patients

Factor	Sub-category	Group – No. of Patients (%)		*p*-value
		Control	Case	
Age group (years)	≤20	2 (8)	1 (4)	0.3673
	21-25	7 (28)	8 (32)	
	26-30	15 (60)	11 (44)	
	>30	1 (4)	5 (20)	
Gravida	Primi	12 (48)	11 (44)	0.7766
	Multi	13 (52)	14 (56)	
(mean± SD)				
Blood pressure	Systolic	117.2±7.09	127.04 ± 6.25	<0.0001*
	Diastolic	75.6±6.98	81.76 ± 5.67	0.0006*

*Significant (p<0.05).

The mean period of gestation during the first blood sampling was 24.56±1.23 and 24.2±1.29 weeks in the control and case groups respectively. The intergroup difference concerning the mean period of gestation was insignificant (p=0.3123).

The mean plasma TAC was significantly higher in the control group (750.02±74.12 µmol/l) when compared to the case group (580.97±168.37 µmol/l) (p<0.0001). The mean uric acid levels were significantly higher in the case group (6.36±1.15 mg/dl) when compared to the control group (4.32±0.48 mg/dl) (p<0.0001).

The effect of group on change in TAC concentration was significant (p<0.0001) and the interaction effect of gravida and group on TAC was insignificant (p=0.2157) (Table 2).

Table 2: Levels of TAC in patients in the study

Gravida	Group – TAC (µmol/l)		*p*-value (int)
	Control	Case	
Primi	742.36±57.46	521.98±195.75	0.2157
Multi	757.10±88.58	627.31±132.68	
p-value	<0.0001*	<0.0001*	

*Significant (p<0.05).
(int) indicates the *p*-value of interaction

During their 3rd trimester, the mean plasma TAC concentration was significantly higher in the case group (856.37±290.09 µmol/l) when compared to the control group (467.7±162.39 µmol/l) (p<0.0001). However, difference in the mean plasma TAC between gravida and groups was insignificant (p=0.7936). An average decrease of 36.91% in the plasma TAC of the control group and an average relative increase of 59% in the plasma TAC in the case group women was observed.
The correlation between TAC, uric acid levels and birth weight in the control group was insignificant. The birth weight and uric acid in case group were highly negatively correlated (Table 3).

Table 3: Correlation between TAC, uric acid, and birth weight

	Control group				Case group			
	Uric acid		Birth weight		Uric acid		Birth weight	
	r	*p*-value	r	*p*-value	r	*p*-value	r	*p*-value
TAC	-0.02[b]	0.9481	-0.11	0.5913	0.11	0.5912	-0.15 [Sp]	0.489
Uric acid	-		0.10 [b]	0.7099	-		-0.85[Sp]	<0.0001*

Abbreviation: TAC, total antioxidant capacity.
*Significant (p<0.05); [b] indicates biserial correlation; [Sp] indicates spearman rank correlation; r indicates correlation coefficient

At the time of delivery, the mean period of gestation was 38.2±1.08 and 34.04±3.8 weeks in the control and case groups respectively, and the difference was significant (p<0.0001).
In terms of delivery, 2 (8%) women from control and 15 (60%) from case group underwent pre-term delivery. The odds of pre-term delivery were 17.25 (95% Confidence Interval (CI): [3.31, 89.97]) times higher for women in the case group than the control group. The association of pre-term delivery and type of uterine perfusion was significant (p=0.0001).
The delivery outcome of the patients concerning the mode of delivery, admission to the neonatal intensive care unit (NICU), birth weight of the infant and still births are represented in Table 4.

Table 4: Delivery outcomes amongst the patients

Factors	Sub-category	Group – No. of Patients (%)		*p* value
		Control	Case	
Mode of delivery	Vaginal	21 (84)	17 (68)	0.1853
	Caesarean	4 (16)	8 (32)	
NICU admission		0	11 (57.89)	-
Still births		0	6 (24)	-
		(mean± SD)		
Birth weight of infants (Kgs)		2.96±0.28	1.73±0.82	<0.0001*

Abbreviation: NICU, neonatal intensive care unit
*Significant (p<0.05)

No patients in the control group developed pre-eclampsia or IUGR, whereas in the case group, 17 (68%) developed pre-eclampsia and 7 (28%) developed IUGR without any pre-eclampsia.

4. Discussion

A particular placental syndrome contributing towards maternal and perinatal morbidity and mortality worldwide is the preeclampsia, characterized by the surge blood pressure during pregnancy [13, 14]. Uterine artery Doppler assessment is a stand-alone test for preeclampsia screening, which had the best predictive value in comparison to the other tests such as increased body mass index, placental growth factor or placental protein 13 having moderate predictive value [2, 15]. Therefore, this study assessed uterine perfusion along with evaluating the TAC, to screen for any abnormality in pregnant women during their second trimester.
The plasma TAC concentration of women in the case group was significantly lower than the control group (p<0.0001). Comparable results were reported in studies conducted by Stepan *et al.* (p<0.05) [16]. Increased antioxidant status along with lipid peroxides with advancing pregnancy has been reported. Since there is placental oxidative stress throughout the pregnancy, there can be a possibility that the antioxidant system acts in retaliation to the changes in oxidative stress to maintain the normal intercellular integrity and function in processes that are susceptible to oxidative stress. Complications in pregnancy such as pregnancy loss, preeclampsia or IUGR may occur due to any disruption in the oxidative stress balance [17].
It was noticed that the gravida status does not affect the TAC values, in both the control and case groups. Therefore, this infers that the antioxidant activity could be more associated to the placental perfusion than the gravida status of a woman.
During the 2nd trimester pregnancy, uric acid levels in the case group was higher when compared the control group. Increased levels of uric acid in preeclampsia cases has been earlier reported, which was in turn correlated with increased maternal and fetal morbidity [18, 19]. However, a significant rise in TAC in the case group women in comparison to the control group during the 3rd trimester pregnancy was noted (p<0.0001). Similarly, increased TAC levels were reported by Hermawan *et al.* (p<0.01) and Le *et al.* (p<0.001) in pregnant women with eclampsia cases when compared to women having a normal pregnancy [20, 21]. There are conflicting reports in literature with respect to the antioxidant levels in connection to the placental syndrome, where some studies show decreased and some show increased circulating antioxidant levels [22-26]. These are likely due to different methods employed in assessing the antioxidant levels, either by assessing the TAC or distinct antioxidative enzymes or by correlating/not correlating the antioxidant levels with that of uric acid, since the endocrine environment of pregnancy influences the uric acid levels [27]. Kharb *et al.* explained that increased TAC (measured by total radical absorption potential method [TRAP]) in preeclampsia was due to higher levels of uric acid in serum [28]. Uric acid contributed nearly 38-47% in measuring TAC by TRAP method. Uric acid antioxidant contributed nearly 60% to the measurement conducted by FRAP. This suggests an indirect evidence of oxidative stress in preeclampsia. But it remains to unknown whether these changes are a cause or consequences of the disease [14]. The TAC and uric acid levels were negatively correlated in the control group and it was positively correlated in the case group. The gestation period of women and the birth weight of infants concerning the case group was lower compared to the control group, which is attributed to placental dysfunction, also affecting the mode of delivery in women [29].
Small sample size, single-centred study and measurement of TAC only via FRAP assay are a few limitations of this study. Future studies overcoming these limitations is vital to understand and determine the exact phenomena and therefore improve the management strategies of such high-risk

pregnancies. In the 2nd trimester pregnancy, an improved predictive value of the uterine artery perfusion should forewarn the obstetrician to provide adequate surveillance of high-risk pregnancies and ensure early interventions.

5. Conclusion
During the 2nd trimester pregnancy, inadequate utero-placental perfusion reduced plasma TAC levels. This shows a scope for therapeutic augmentation of antioxidant levels in preeclampsia and measuring the antioxidant status may have possible diagnostic, pathophysiologic, therapeutic implications not only in preeclampsia, but also in other diseases with oxidative stress.

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Conflict of Interest
The authors declare that there are no conflicts of interest

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6. References
1. Browne VA, Julian CG, Toledo-Jaldin L, Cioffi-Ragan D, Vargas E, Moore LG. Uterine artery blood flow, fetal hypoxia and fetal growth. Philos Trans R Soc Lond B Biol Sci 2015;370:20140068.
2. Ridder A, Giorgione V, Khalil A, Thilaganathan B. Preeclampsia: The relationship between uterine artery blood flow and trophoblast function. Int J Mol Sci 2019;20:3263.
3. Upadya M, Rao ST. Hypertensive disorders in pregnancy. Indian J Anaesth 2018;62:675-681.
4. Berhe AK, Kassa GM, Fekadu GA, Muche AA. Prevalence of hypertensive disorders of pregnancy in Ethiopia: A systemic review and meta-analysis. BMC Pregnancy Childbirth 2018;18:34.
5. Wu F, Tian FJ, Lin Y. Oxidative Stress in Placenta: Health and Diseases. Biomed Res Int 2015;2015:293271.
6. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med 1999;222:222-235.
7. Poston L, Raijmakers MTM. Trophoblast oxidative stress, antioxidants and pregnancy outcome: A review. Placenta 2004;25:S72-S78.
8. Marseglia L, D'Angelo G, Manti S, Arrigo T, Barberi I, Reiter RJ *et al*. Oxidative stress-mediated aging during the fetal and perinatal periods. Oxid Med Cell Longev 2014. Article ID 358375.
9. Chien PF, Arnott N, Gordon A, Owen P, Khan KS. How useful is uterine artery Doppler flow velocimetry in the prediction of preeclampsia, intrauterine growth retardation and perinatal death? An overview. BJOG 2000;107:196-208.
10. Papageorghiou AT, Yu CK, Bindra R, Pandis G, Nicolaides KH. Multicenter screening for preeclampsia and fetal growth restriction by transvaginal uterine artery Doppler at 23 weeks of gestation. Ultrasound Obstet Gynecol 2001;18:441-449.
11. Mannaerts D, Faes E, Cos P, Briedé JJ, Gyselaers W, Cornette J *et al*. Oxidative stress in healthy pregnancy and preeclampsia is linked to chronic inflammation, iron status and vascular function. PLoS One 2018;13:e0202919.
12. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem 1996;239:70-76.
13. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: A systematic review. Eur J Obstet Gynecol Reprod Biol 2013;170:1-7.
14. Severens-Rijvers CAH, Al-Nasiry S, Vincken A, Haenen G, Winkens B, Ghossein-Doha C *et al*. Early-pregnancy circulating antioxidant capacity and hemodynamic adaptation in recurrent placental syndrome: An exploratory study. Gynecol Obstet Invest 2019;84:616-622.
15. Townsend R, Khalil A, Premakumar Y, Allotey J, Snell KIE, Chan C *et al*. Prediction of pre-eclampsia: review of reviews. Ultrasound Obstet Gynecol 2019;54:16-27.
16. Stepan H, Heihoff-Klose A, Faber R. Reduced antioxidant capacity in second-trimester pregnancies with pathological uterine perfusion. Ultrasound Obstet Gynecol 2004;23:579-583.
17. Furukawa S, Nakajima A, Sameshima H. The longitudinal change of extracellular antioxidant status during pregnancy using an electron spin resonance method. J Matern Fetal Neonatal Med 2016;29:2994-2999.
18. Rajalaxmi K, Radhakrishna N, Manjula S. Serum uric acid level in preeclampsia and its correlation to maternal and fetal outcome. Int J Biomed Res 2014;5:22-24.
19. Essiben F, Itembe O, Foumane P, Nguefack MT, Eko FE. Blood uric acid level as a marker of increased risk of eclampsia in severe pre-eclamptic patients: A cross-sectional study in two tertiary hospitals of Yaoundé, Cameroon. Health Sciences and Diseases 2016;17:7-11.
20. Hermawan M, Mose JC, Madjid TH. Correlation of total antioxidant capacity measured by ferric reducing ability of plasma (FRAP) assay with the severity of preeclampsia. Indonesian Journal of Obstetrics and Gynecology 2011;35:155-160.
21. Le TM, Nguyen LH, Phan NL, Le DD, Nguyen HVQ, Truong VQ *et al*. Maternal serum uric acid concentration and pregnancy outcomes in women with pre-eclampsia/ eclampsia. Int J Gynaecol Obstet 2019;144(1):21-26.
22. Kirbas A, Daglar K, Gencosmanoglu G, Yilmaz Z, Timur H, Inal Z *et al*. Total oxidative and anti-oxidative status, and Adamts-12 levels in placenta previa and early-onset severe preeclampsia. Pregnancy Hypertens 2016;6:295-299.
23. D'Souza V, Rani A, Patil V, Pisal H, Randhir K, Mehendale S *et al*. Increased oxidative stress from early pregnancy in women who develop preeclampsia. Clin Exp Hypertens 2016;38:225-232.
24. D'souza JMP, Harish S, Pai VR, Shriyan C. Increased oxidatively modified forms of albumin in association with decreased total antioxidant activity in different types of hypertensive disorders of pregnancy. Indian J Clin Biochem 2017;32:200-206.
25. Nikolic A, Cabarkapa V, Novakov Mikic A, Jakovljević A, Stosic Z. Ceruloplasmin and antioxidative enzymes in pre-eclampsia. J Matern Fetal Neonatal Med 2016;29:2987-2993.
26. Keshavarz P, Nobakht M, Gh BF, Mirhafez SR, Nematy M, Azimi-Nezhad M *et al*. Alterations in lipid profile, zinc and copper levels and superoxide dismutase activities in normal pregnancy and preeclampsia. Am J Med Sci 2017;353:552-558.
27. Quiñones Galvan A, Natali A, Baldi S, Frascerra S, Sanna G, Ciociaro D *et al*. Effect of insulin on uric acid excretion in humans. Am J Physiol 1995;268:E1-E5.
28. Kharb S. Total free radical trapping antioxidant potential in pre-eclampsia. Int J Gynaecol Obstet 2000;69:23-26.
29. Krishna U, Bhalerao S. Placental insufficiency and fetal growth restriction. J Obstet Gynaecol India 2011;61:505-511.