Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 11
ISSN: 2321–3647(online)

Evaluation of Lipid Peroxidation, Protein Carbonyl Content and Total Antioxidant Status in Pre and Post-Delivery of Women with Preeclampsia

Vanishree Bambrana[1], C D Dayanand*[2], R Sheela[3], Pradeep Kumar Vegi[4]
1. Dept of Biochemistry, Sri Devaraj Urs Medical College, Kolar, Karnataka 563 101.
2. Dept of Biochemistry Head of Allied Health Sciences Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka 563 101.
3. Dept of Obstetrics and Gynecology Sri Devaraj Urs Medical College, Karnataka 563101.
4. Dept of Biochemistry Senior Research Fellow, Dept. of Cell Biology & Molecular biology Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka 563101.

ABSTRACT

Preeclampsia is a metabolic syndrome and a major cause of maternal, fetal and neonatal morbidity and mortality that amounts to 10% of the pregnancy complications. To determine Malondialdehyde, protein carbonyl and total antioxidant status in preeclampsia and normal pregnant women during pre and post labour within 48 hours. A prospective case control study (n= 60), preeclampsia (n=30) and normal pregnant women (n=30) during pre and post labour within 48 hours. 3 ml of blood samples were collected during pre and post labour of normal pregnant and preeclampsia. MDA, Protein Carbonyl Content and Total Antioxidant Status were measured using spectrophotometric method. Mean ± SD were determined by using student "t" test. The Mean ± SD values of MDA µmol/L (4.7 ±1.8), Protein Carbonyls (168.9±70.5) nmol/, Total Antioxidant Status mmol/L (537 ± 451) in normal pregnant during pre-delivery and MDA µmol/L(2.4 ±0.1), Protein Carbonyls (169±67.2)nmol/L , Total Antioxidant Status mmol/L (634.3 ± 241.2) post-delivery presented. Similarly Mean ± SD values of MDA µmol/L (10.1 ±6.4), Protein Carbonyls (159±123.2)nmol/L , Total Antioxidant Status mmol/L (506.7 ± 287.6) in preeclampsia pre delivery and (6.1±6.1), (98.8±36.8), (680±362.3) post-delivery respectively. A well-known aspect in preeclampsia with increased concentrations of oxidative stress and decreased TAS. However the trend of increased TAS and declined oxidative stress during post labour of normal pregnant and preeclampsia which plays a significant role in pathophysiology of preeclampsia.

Keywords: Preeclampsia, Before delivery, After delivery, Protein carbonyl content

*Corresponding Author Email cd8905@yahoo.co.in
Received 06 September 2014, Accepted 11 September 2014

Please cite this article as: Dayanand CD *et al.*, Evaluation of Lipid Peroxidation, Protein Carbonyl Content and Total Antioxidant Status in Pre and Post-Delivery of Women with Preeclampsia. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Preeclampsia is a clinical condition characterized by hypertension and proteinuria. This obstetric complications leading to fetal uterine growth restriction, preterm delivery, maternal and fetal morbidity and mortality[1-2].It occurs in 5-7 % of the pregnancies worldwide.[3]The incidence is still higher in India of around 8-10%.[4]As per the World Health Report the maternal mortality during pregnancy and puerperium is around 12 %.In developing countries, 17% of direct obstetric deaths are as a result of hypertension[5]. The mortality rate of preeclampsia in the developing and developed countries varies approximately eight hundred women die from pregnancy and child birth related complications around the world every day[6]. Although aetiology of preeclampsia is not clear, maternal symptoms are of secondary to endothelial dysfunction causes imbalance to the oxidant and antioxidants ratio in preeclampsia. Propagation of free radicals are known for generation of oxidative stress and thus play a prominent role in preeclampsia. Therefore measurement of indicators of oxidative stress helps to understand an imbalance of oxidants and antioxidants. In the current research, an attempt is made to report the oxidative stress markers for lipid peroxidation as MDA and Protein oxidation as carbonylated proteins in terms of protein carbonyls and also plasma total antioxidant status in pre and post labour of preeclampsia in comparison to normal pregnant group.

MATERIALS AND METHOD

Pregnant women in the third trimester attended to Department of Obstetrics and Gynecology, R.L. Jalappa Hospital and Research Center, Kolar, Karnataka were enrolled for the prospective case control study. Preeclampsia was defined as systolic and diastolic blood pressure greater than 140 mm of Hg and 90 mm of Hg, with significant proteinuria (>300 mg per 24 hr). From this total subjects 30women were clinically diagnosed as Preeclampsia and another 30 was normal pregnant woman were included in the study after obtaining the University Ethical Committee approval and patient information consent. After performing physical examination and obtaining the complete patient history, three ml of blood samples were drawn into Heparinized vaccutainer at pre and post-delivery of women with preeclampsia and normal pregnant women respectively. The content of the tubes were mixed and centrifuged for 15 min at 4000rpm to separate the clear plasma that was used for quantification of Malondialdehyde, Protein carbonyl content and Total antioxidant status. Malondialdehyde (MDA) a marker of lipid peroxidation was measured in plasma by spectrophotometrically using standard as tetra methoxy propane (TMP) as thiobarbituric acid reactive substances on precipitation of proteins with trichloroacetic acid as per

the method described by Sinnbhuber RO et al[7]. Protein carbonyls a marker of protein oxidation was measured in plasma by spectrophotometrically using internal standard oxidized BSA and reduced BSA according to method mentioned by H. Busset al[8] .Oxidized BSA containing additional carbonyls was prepared by reacting (50mg/ml) with hypochlorous acid to final concentration of 5mM. Reduced BSA is prepare by adding 0.1 g sodium borohydride to a solution of 0.5g BSA in 100ml phosphate buffer saline. After 30 minutes, this solution was brought to neutral pH with 2M HCl and then subjected for dialysis. Using reduced BSA from the dialysate and oxidized BSA obtained from the above step used as as standard maintaining constant protein concentration (4mg/ml).Thus prepared standards used during determination of protein carbonyl content by Levin et al[9] method. Plasma Total antioxidant status determined by means of ferric reducing ability of plasma that was measured spectrophotometrically according to the method described by Benzie I F et al[10].The unpaired t test was used to assess the statistical significance of difference between the study groups. A probability level of <0.05 considered as statistically significant. Results are reported as mean standard ± deviation.

RESULTS AND DISCUSSION

Malondialdehyde as lipid peroxidation marker, Protein carbonyl content as a marker of Protein oxidation in oxidative stress and Total Antioxidant status were measured in Preeclampsia and normal pregnant during pre and post labour within 48 hours. Table 1 shows the concentration of MDA, Protein carbonyl content and total antioxidant status in healthy pregnant women and preeclampsia during pre-labour. The results between two groups evinced an observation of increased MDA level in preeclampsia group (10.1±6.4) in comparison to normal pregnancy during pre-labour (4.7±1.8) with p value <0.05 as significance. Study clearly indicates that there are no significant results with respect to protein carbonyls and total antioxidant status between normal pregnancy and preeclampsia during post-delivery. The levels of MDA, Protein carbonyl content and total antioxidant status in normal pregnant women and preeclampsia during post-delivery were shown in Table 2. The decreased levels of protein carbonyl content in preeclampsia (98.8±36.8) compared to the normal pregnancy (169±67.2).MDA levels increased in preeclampsia (6.1±6.1) after delivery when compared to the normal pregnancy (2.4±1.0) which is highly significant with the p value of <0.005.But no difference is observed with respect to Total Antioxidant Status between the two groups during post-delivery. The striking observation is that increasing trend of total antioxidant status in the two groups after delivery within 48 hours with concomitant decrease of lipid peroxidation. Oxidative stress propagates

generation of reactive oxygen species against buffering capacity of antioxidants. The altered homeostasis implicated in the onset of atherosclerosis, cancers, pre-eclampsia, and many other diseases. Oxidative stress markers are also increased in the decidua, placenta, and other maternal tissues[11]. Oxidative stress as a potential indicator in preeclampsia has been studied, MDA is one of the first biomarkers of lipid peroxidation found to elevate in the plasma of women with preeclampsia[12]. Similarly we observed significantly increased MDA levels in preeclampsia .This observation holds good and supported by Petra LM et al[13]. He has explained the mechanism of increase of MDA during cell turn over and decline in antioxidants free radical trapping mechanism. The current study emphasis two fold increase of MDA as a marker of oxidative stress in preeclampsia compared to the control group and nearly fifty percent reduction of MDA was seen between two groups during before and after delivery indicates consistent elevation of MDA. This proves a radical increase in MDA level in control and further increase in Preeclampsia groups as supported by other studies[14].Therefore serum MDA measurement can be used as s prognostic indicator of oxidative stress that also raise an alarm of need of antioxidant supplementation during preeclampsia. Increase in total antioxidant capacity is seen in preeclampsia and healthy pregnant women after delivery when compared to before delivery which might be due to compensatory regulation in response to the oxidative stress[15].There was no difference in Protein carbonyl content between preeclampsia and healthy pregnant women before delivery. As observed in the present study, increased protein carbonyl content in normal pregnancy as well as in preeclampsia was also reported by zusterzeelet al[16]. Increase in MDA is seen in normal pregnancy that generates an imbalance in homeostasis of oxidant and antioxidants. This elevation further aggravates preeclampsia which might result in endothelial damage leading to increased diastolic blood pressure[17] and after delivery MDA level in preeclampsia has not attain to the level of MDA levels in normal pregnancy within forty eight hours. That shows higher level of oxidative stress persists after deivery[18]. In the present study we found that baseline elevation of MDA levels were seen in both the groups with a proportionate variation. The study results also emphasis the reduction in MDA levels in both the groups after delivery with increased TAS level compared to pre labour where it is associated with elevated oxidative stress in preeclampsia. This inverse relationship clearly demonstrates the homeostasis of oxidative stress and antioxidant status. Increased lipid peroxidation and decreased total antioxidant status observed in preeclampsia in comparison to control group in pre delivery. Significant reduction of MDA, protein carbonyl were seen in control and preeclampsia in post-

delivery compared to pre delivery. However protein carbonyl is unaltered in control group but decreased in preeclampsia before and after delivery.

Table 1: Circulating levels of plasma MDA, Protein carbonyl content and total antioxidant status in healthy pregnant women and pregnant women with preeclampsia during pre-labour.

Parameters	pregnant women Pre Labour (Mean±SD)	Pre-eclampsia Pre Labour (Mean±SD)	p value
Protein carbonyl (nmol/L)	168.9±70.5	159±123.2	>0.05
MDA (µmol/L)	4.7±1.8	10.1±6.4	<0.05*
TAS(mmol/L)	537±451	506.7±287.6	>0.05

* P value <0.05 is considered as statistically significant

** Indicates Highly Significant

Table 2: Circulating levels of plasma MDA, Protein carbonyl content and Total antioxidant status in healthy pregnant women and pregnant women with preeclampsia during post labour

Parameters	pregnant women post labour (Mean±SD)	Pre-eclampsia Post labour (Mean±SD)	p value
Protein carbonyl (nmol/L)	169±67.2	98.8±36.8	<0.05*
MDA(µmol/L)	2.4±1.0	6.1±6.1	<0.005**
TAS(mmol/L)	634.3±241.2	680±362.3	>0.05

*P value <0.05 is considered as statistically significant

** Indicates Highly Significant

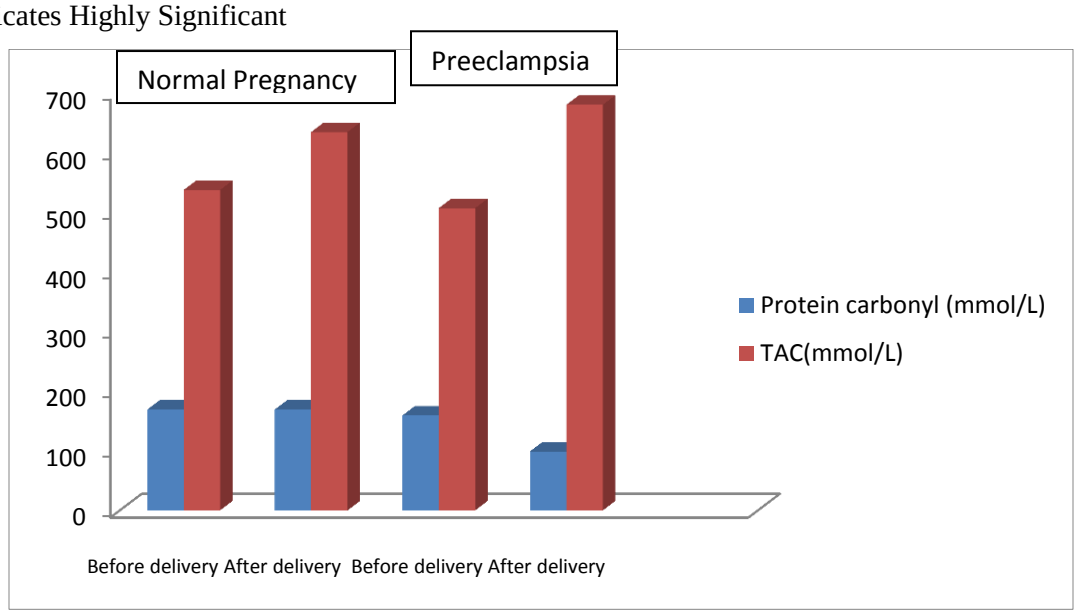

Figure 1: Showing the levels of Protein carbonyl content, TAS between two groups during pre and Post Labour.

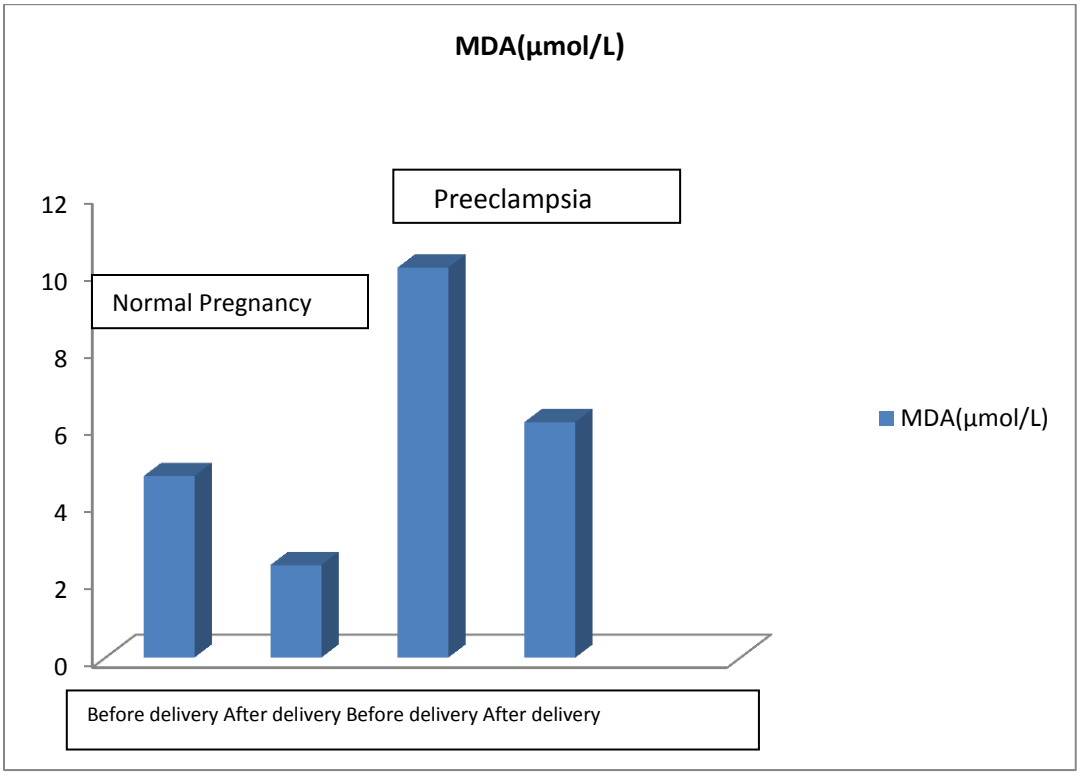

Figure 2: Showing the levels of MDA between the two groups during pre and post labour

CONCLUSION

A well-known aspect in preeclampsia with increased concentrations of oxidative stress and decreased TAS. However the trend of increased TAS and declined oxidative stress during post labour of normal pregnant and preeclampsia which plays a significant role in Pathophysiology of preeclampsia.

REFERENCES

1. Cindrova-Davies T. Gabor Than award lecture 2008: preeclampsia-From placental Oxidative stress to maternal endothelial dysfunction.Placenta2009;30:55-65.
2. Von Dadleszen, P.Mageela, Taylor EL, Muir JC, Stewart SD, et al. Maternal hypertension and neonatal outcome among small for gestational age infants. ObstectGyneco 2005; 106:335-39.
3. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap DJ, Wenstrom SY. Williams Obstetrics.23rd Edition. McGraw Hill Medical Publishing Division 2010; section VII, 34 : 706-56.
4. Mutlu TU, Ademoglu E. Imbalance between lipid peroxidation, antioxidant status in preeclampsia. GynecolObstet Invest 1998; 46 : 37-40.

5. Maternal mortality in 2005: estimates developed by WHO, UNICEF, UNIFPA and the World Bank, Geneva, World Health Organization, 2007.
6. World Health Organization Fact Sheet, May 2012.
7. Sinnbhuber RO, Yu IC, Yutec. Food Res 1958; 23:620.
8. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A, Ahn B. Shalteil S, Stadtman ER. Determination of carbonyl content of oxidatively modified proteins. Methods Enzymol. 1990; 186:464-78.
9. HendrikjeBuss, Timothy P, Chan, Karl B, Sluis, NeilM, Domigan, Christine C, Winterbourn. Protein carbonyl measurement by a sensitive ELISA method. Free radical Biology& Medicine 1997; 23:361-66.
10. BenziIF, StrainJJ. The ferric reducing ability of plasma as a measure of "antioxidant power" the FRAP assay. AnnalBiochem1996; 239:70-76.
11. Hubel CA, Roberts JM, Taylor RN, Musci TJ, Rogers GM, Mclaughlin MK. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. Am J ObstetGynecol 1989; 161: 1025–34.
12. J.B. Sharma, A. Sharma, A. Bahadur, N. Vimala, A. Satyam, S. Mittal. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. International Journal of Gynecology and Obstetrics 2006; 94: 23-27.
13. Petra L.M. Zusterzeel, Theo P.J.Mulder, Wilbert H.M, Peters, Sheila A, Wiseman and Eric A.P.Steegers. Plasma protein Cabonyls in Non-pregnant, Healthy Pregnant and Preeclamptic women. Free Rad Res 2000; 33:471-76.
14. OzlemKaracy, DenizKarcaaltincaba, DuyguSahin, SerdarYalvac, MescutAkyol, Omerkandemir, Nilln. A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24-36 weeks of gestation. J.Diab.Res; 2010; 89:231-38.
15. MohdSuhail, MohdFaizulsuhail, Hina Khan. Alterations in antioxidant and pro-oxidant balance in preeclampsia-impact on erythrocyte osmotic fragility. BiochemiaMedica 2008; 3:331-41.
16. Zusterzeel PL, Rutten H, Roelofs HM, Peters WH, Steegers EA. Protein carbonyls in deciduas and placenta of preclamptic women as markers for oxidative stress. Placenta 2001;22:213-19.

17. J.B. Sharma, A. Sharma, A. Bahadur, N. Vimala, A. Satyam, S. Mittal. Oxidative stress markers and antioxidant levels in normal pregnancy and Pre-eclampsia. Int J GynecolObstet2006; 94:23-27.
18. Ozan H, Ilcol Y, Kimya Y, Cengiz C, Ediz B. Plasma antioxidant status and lipid profile in non –gravida women with history of preeclampsia. J Obstet Gynecol Res 2002;28:274-79.