ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Role Of Oxidative Stress And Antioxidant In Preeclampsia: A Study In Rural Population.

Ranjit S. Ambad[1], Roshan Kumar Jha[*1], Nandkishor Bankar[2], Minal Kalambe[3], Deepti Shrivastava[4]

[1]Department of Biochemistry Datta Meghe Medical College, Shalinitai Meghe Hospital & Research Centre, Hingana, Nagpur, Maharashtra, India
[2]Department of Microbiology Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Wardha-442001, India
[3]Department of OBGY Datta Meghe Medical College, Shalinitai Meghe Hospital & Research Centre, Hingana, Nagpur, Maharashtra, India
[4]Department of OBGY Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Wardha-442001, India

Article History:

Received on: 22 Mar 2020
Revised on: 22 Apr 2020
Accepted on: 22 May 2020

Keywords:

MDA,
SOD,
HO,
ROS,
NADH,
NADPH,
GR,
pre-eclampsia

ABSTRACT

Pre-eclampsia is well known complication of gestation which generally occurs around 20 weeks of pregnancy; it is linked with large maternal and fetal mortality and morbidity. The disease comes with new-onset hypertension and proteinuria in the mother, which can further progress to dysfunction of multiple organs, and includes hepatic, renal and cerebral disease. To estimate malondialdehyde (MDA), glutathione reduced, superoxide dismutase and catalase in pregnant women with pre-eclampsia. Role of malondialdehyde, antioxidant and their relationship were estimated using different method. Preeclamptic group was compared with normal pregnant women and another group of pregnant women administered with supplementation of Vitamins. In present study we found decrease in levels of reduced glutathione level in pre-eclamptic group as compared to normal and supplementation group. The levels of superoxide dismutase was found to be increased in present study in pre-eclamptic group as compared to normal pregnant women and pregnant women with supplementation and the differences were highly significant. In present study where antioxidants level was compared with pre-eclamptic group and group with supplementation the markedly significant variations were seen. Our finding on role of antioxidant in preeclampsia when compared with supplementation of Vitamins and normal pregnant women the result showed beneficial affect with the significant differences.

*Corresponding Author

Name: Roshan Kumar Jha
Phone: 9890959395
Email: rossssan47@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2465

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

Preeclampsia is a common complication of gestation. It may occur around the 20^{th} week of pregnancy; it may mainly lead to maternal and foetal mortality and morbidity. Preeclampsia is also one of the most extreme pregnancy complications, and the disease's pathophysiology is not well known. The potential role of the genetic and immune pathways in preeclampsia etiology has attracted increased attention (Yadav *et al.*, 2018).

The disease comes with new-onset of hyperten-

sion and Proteinuria in the pregnant women, which can further progress to the dysfunction of multiple organs, and includes hepatic, renal and cerebral diseases if the placenta along with foetus is not delivered. Dysfunction of maternal endothelial due to circulating factors of foetal origin from the placenta is a hallmark of preeclampsia. This threatening disease may cause maternal comorbidities, such as chronic kidney disease, hypertension and obesity; a family history of preeclampsia, multiple pregnancies; and previous preeclampsia or intrauterine foetal growth restriction are accompanied risk factors (Ambad and Dhok, 2019).

Preeclampsia generally affects 0.4% to 2.8% of pregnancies in developed countries and much more in developing countries. A result of 8370000 cases of preeclampsia is seen per year worldwide (Chamy *et al.*, 2006). In accordance to the criteria of the International Society of the Study of Hypertension in Pregnancy, the ideal definition is a diagnosis of pregnancy-induced hypertension (diastolic blood pressure >90 mm Hg) occurring after a week 20 of pregnancy with Proteinuria (either $\geq$300 mg protein per day or a urinary protein/creatinine ratio $\geq$30 mg/mmol) (Brown *et al.*, 2001). When patients come along with liver dysfunction, thrombocytopenia, and hemolysis, they are considered as having HELLP syndrome (i.e., haemolysis, elevated liver enzymes, low platelets) (Curtin and Weinstein, 1999). Nitrosative stress is a focal point for many contributing factors that could eventually contribute to clinical manifestations of pregnancy-induced hypertension (Gaikwad *et al.*, 2017).

Although the definitions focus on these simply measured clinical parameters, preeclampsia must be renowned as a multisystem disorder, which unreliably may affect the brain, lungs, kidney, and liver. To date, there is no medication for preeclampsia, and in the severe condition, it needs premature labour induction, that carries with it, the inherent threats for premature neonates (Aouache *et al.*, 2018).

The incidence of preeclampsia in India is reported to be between 8 to10 percent among pregnant women. According to a study, the occurrence of hypertensive conditions of pregnancy was around 7.8% with preeclampsia in 5.4% of the study population in India (Ambad and Dhok, 2019).

Oxidative stress is known as disproportion between the formation of oxidative substances and the inborn antioxidants that make up the endogenous defence system. Oxidative substances are generally free oxygen radicals and peroxides that routinely form in small amounts (Buonocore *et al.*, 2010). It has been recognised as a main factor causing endothelial dysfunction in preeclampsia, though evidence supporting this hypothesis remains unreliable (Llurba *et al.*, 2004). Oxidative stress is widely associated with abortive reproductive performance, including infertility, miscarriage, diabetes-related congenital abnormalities, preeclampsia sickle cell anaemia and sickle cell disease (Baliga *et al.*, 2017; Wasnik, 2017). Maternal obesity is a tough risk factor for preeclampsia, and in a latest study concluded oxidative stress in the oocytes of obese animals formerly in gestation as well as in early-stage embryos (Poston *et al.*, 2011).

Antioxidants are molecules that prevent the oxidation caused due to oxidative substances, and they consist of two sub-groups; enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants are superoxide dismutase (SOD), hemoxygenase (HO), and catalase. SOD's enzymatic activity catalyzes the dismutation of superoxide ($O^{-}{}_{2}$) into oxygen and hydrogen peroxide (H_2O_2) (Mccord and Fridovich, 1988).

Non-enzymatic antioxidants are glutathione, thioredoxin, NADH, and NADPH. Glutathione is a vital endogenous antioxidant that aids several functions, such as neutralization of free radicals and ROS (Pompella *et al.*, 2003).

This study was aimed to analyse in depth the latent role of oxidative stress as a mechanism underlying endothelial damage in preeclampsia, the pregnant woman's proneness to the disease and decrease in susceptibility of disease in women with supplementation of vitamins. To this end, suggestive markers of lipoperoxidation and protein oxidation and changes in antioxidant defence systems were estimated in blood samples.

Aims And Objectives

The present study was undertaken in the Department of Biochemistry and Department of Obstetrics and Gynaecology at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Meghe, Wardha, in collaboration with Datta Meghe Medical College Hingana, Nagpur, Maharashtra India with the following aims and objectives: -

Aim of Study

To estimate the role of oxidative stress and antioxidant in preeclampsia.

Objectives of study

1. To estimate malondialdehyde (MDA), glutathione reduced, superoxide dismutase and catalase in pregnant women with preeclampsia.

Table 1: MDA & antioxidants level in normal pregnant women & pregnant women with preeclampsia.

MDA & Antioxidants levels	Group I	Group III	'P' Value
Malondialdehyde	5.18±0.15	8.21±0.55	P<0.0001
Glutathione reductase	39.25±2.06	32.10±1.50	P<0.0001
superoxide dismutase	103.2±4.52	121±5.58	P<0.0001
Catalase	523±25.31	499±19.33	P<0.0001

Table 2: MDA & antioxidants level in pregnant women with supplementation of Vit A, C& E, & pregnant women with preeclampsia.

Serum minerals	Group II	Group III	'P' Value
Malondialdehyde	4.89±0.25	8.21±0.55	P<0.0001
Glutathione reductase	43.25±3.16	32.10±1.50	P<0.0001
superoxide dismutase	110.12±5.12	121±5.58	P<0.0001
Catalase	522±28.14	499±19.31	P<0.0001

Table 3: MDA & antioxidants level in normal pregnant women and pregnant women with supplementation of Vit A, C & E.

Serum minerals	Group I	Group II	'P' Value
Malondialdehyde	5.18±0.15	4.89±0.25	P<0.0001
Glutathione reductase	39.25±2.06	43.25±3.16	P<0.0001
superoxide dismutase	103.2±4.52	110.12±5.12	P<0.0001
Catalase	523±25.31	522±28.14	P=0.8522

2. Find out correlation of malondialdehyde (MDA), glutathione reduced, superoxide dismutase and catalase in preeclampsia in comparison with normal pregnant women and pregnant women with supplementation of vitamins.

MATERIALS AND METHODS

Study Area

The present study was undertaken in the Department of Biochemistry of Jawaharlal Nehru Medical College, and AVBRH (Datta Meghe Institute of Medical Sciences) Sawangi, Meghe, Wardha in collaboration with Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Hingana, Nagpur, Maharashtra India.

Study Population

150 subjects were included in the study and were grouped as under.

Group I

50 normal pregnant subjects.

Group II

50 subjects with supplementation of Vitamin E, Vitamin C & Vitamin A in diet.

Group III

50 subjects with gestational age between 32 and 36 weeks with mild/severe preeclampsia.

(Blood pressure—>140/90 mmHg, with Proteinuria, with or without pathological oedema)

Study Type

Cross sectional interventional study

Selection Of The Patients

150 pregnant women were taken with following inclusion and exclusion criteria.

Inclusion criteria

1. Pregnant women within the age of 20-40
2. Pregnant women with Newly diagnosed hypertension

3. Pregnant women with Proteinuria (excretion of 300 mg or more in 24-hour collection)
4. Pregnant women with 20 weeks of gestation
5. Pregnant women with HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome can also be included
6. Pregnant women diagnosed with preeclampsia.
7. The mother who are suspected having hypothyroidism (Clinical sign and symptoms) were included in the study. Mothers who were residing in study area at least 10 years or more than 10 years were be included in study.

Exclusion criteria

1. Unwilling participant
2. Pregnant women without preeclampsia or HELLP syndrome.
3. Pregnant women suffering from gestational and essential hypertension, thyroid disorder (clinical or subclinical), immunosuppressed and any endocrine problems Were excluded from study. Those who were more than 35 was excluded from study. Those mothers who are self-medicated and do not follow the instructions were also excluded from study. Mother who was residing in study area less than 10 years was excluded from study.

Sample Processing

For the bio- chemical parameters to be analysed, blood sample was collected from the anticubital vein avoiding venostasis in all subjects. 5 ml of venous blood was drawn from the subject and control after written and informed consent, in dry disposable syringe under aseptic conditions and was transferred to a sterile EDTA vial for biochemical analysis.

Biochemical Investigations

Parameters were assessed immediately within 48 hours using following method Oxidative stress was assessed by quantifying thiobarbituric acid (TBA) reactivity as MDA in a spectrophotometer. The TBAA test for the plasma MDA concentrations was performed using the method described by H.H. Draper. (Koracevic, 2001) Antioxidants levels were estimated using ELISA method. Glutathione reductase were analyzed by -ELISA Method (Faizal *et al.*, 2017). Uperoxide dismutase were analyzed by -ELISA Method (Maarten *et al.*, 2020) and Catalase activity were measured by Spectro-photometric assay (Góth, 1991).

Statistical Analysis

Data collected was entered into Microsoft Excel Worksheet and statistically analysed by using SPSS (Statistical Package for Social Sciences) version 20. For quantitative data mean, standard mean, standard deviation, t-test and Karl Pearson's Coefficient of Correlation were calculated. P value < 0.05 (0.01) will be considered as statically significant (highly significant) at 95% confidence interval.

Ethical Consideration

Informed and written consent (Marathi and English) was taken from each subject before collecting data and blood sample. Only those individuals, who volunteer to participate in the study, was included and the data was kept confidential. The study was not impose any burden on the subjects and the Institute, therefore the study is ethically justified. The proposed study was undertaken subject to approval by Institutional Ethical Committee.

RESULTS AND DISCUSSION

Levels of MDA & Antioxidants in control and pre-eclamptic women are seen in Table 1. Analysis of MDA & Antioxidants level found that mean values of malondialdehyde, reduced glutathione, superoxide dismutase, and catalase were 5.18±0.15, 39.25±2.06, 103.2±4.52, and 523±25.31 in control group and 8.21±0.55, 32.10±1.50, 121±5.58, and 499±19.33 women with preeclampsia respectively. The levels of MDA & super oxidase dismutase were increased significantly (< 0.0001) in pre-eclamptic group as compared to control group. The levels of reduced glutathione & catalase were decreased significantly (< 0.0001) in pre-eclamptic group as compared to control group.

Levels of MDA & Antioxidants in pregnant women with supplementation and pre-eclamptic women are seen in Table 2. Analysis of MDA & Antioxidants level found that mean values of malondialdehyde, reduced glutathione, superoxide dismutase, and catalase were 4.89±0.25, 43.25±3.16, 110.12±5.12, and 522±28.14 in women with supplementation of Vit A, C & E and 8.21±0.55, 32.10±1.50, 121±5.58, and 499±19.33 women with preeclampsia respectively. The levels of MDA & super oxidase dismutase were increased significantly (< 0.0001) in pre-eclamptic group as compared to women with supplementation of Vit A, C & E. The levels of reduced glutathione & catalase were decreased significantly (< 0.0001) in pre-eclamptic group as compared to women with supplementation of Vit A, C & E.

Levels of MDA & Antioxidants in normal pregnant

women & pregnant women with supplementation are seen in Table 3. Analysis of MDA & antioxidants level found that mean values of malondialdehyde, reduced glutathione, superoxide dismutase, and catalase were 5.18±0.15, 39.25±2.06, 103.2±4.52, and 523±25.31 in normal subjects and 4.89±0.25, 43.25±3.16, 110.12±5.12, and 522±28.14 in women with supplementation of Vit A, C & E respectively. The levels of MDA showed significant (< 0.0001) decrease in women with supplementation of Vit A, C & E as compared to normal pregnant women. The levels of reduced glutathione & superoxide dismutase were increased significantly (< 0.0001) in women with supplementation of Vit A, C & E as compared to normal pregnant women. While there were no significant differences seen in catalase levels but the level were decreased in women with supplementation of Vit A, C & E as compared to normal pregnant women.

In present study done in rural population, result shows that the levels of malondialdehyde increased very significantly in pre-eclamptic group as compared to normal pregnant females and further in comparison between pregnant women with preeclampsia and pregnant women with supplementation of Vitamins A, C, & E, elevated levels of malondialdehyde level was seen in pre-eclamptic group as compared to group with supplementation and the differences were very highly significant. The elevation signifies on-going lipid peroxidation in preeclampsia and may be hall mark of oxidative stress. This result correlates well with several studies undertaken at numerous institutes. Indian, studies conducted by Gohil et al, (Gohil *et al.*, 2011) Adiga, Patil et al. (Patil *et al.*, 2007) and Krishna Menon S (Menon *et al.*, 2007) at different areas showed significant elevations in the levels of malondialdehyde in preeclampsia compared to pre-eclamptic pregnancy and non-pregnant females. Other study in world, conducted by different researchers Llurba et al, (Llurba *et al.*, 2004) and Chamy et al. (Miller *et al.*, 1993) have conclude the significant increased MDA levels in preeclampsia as compared to normal pregnancy. This is similar to our findings.

Studies on antioxidant status in preeclampsia have revealed mixed outcomes. In accordance to study done by Poston et al. concluded mixed result whether early supplementation with vitamins C and E in women at risk of preeclampsia is beneficial, but these trials have shown no evidence that these supplements can prevent preeclampsia. Some show decrease in antioxidant enzymes (due to consumption) and some show increase in antioxidant enzymes (as a compensatory mechanism). In present study we found decrease in levels of reduced glutathione level in pre-eclamptic group as compared to normal and supplementation group. The result of present study is somehow similar to the study done by Gohil et al (Gohil *et al.*, 2011).

The levels of superoxide dismutase were found to be increased in present study in pre-eclamptic group as compared to normal pregnant women and pregnant women with supplementation and the differences were highly significant. Increased level of superoxide dismutase was also found in Sharma et al study and Krishna Menon et al. study (Menon *et al.*, 2007). These elevations may be due to an adaptive response to counter the effect of increase in oxidative stress.

Small intervention made in the study to overcome the hypothesis results in a beneficial effect of vitamins supplementation when compared with both pre-eclamptic and normal pregnant women. In present study where antioxidants level was compared with pre-eclamptic group and group with supplementation the markedly significant variations were seen. And again, when the levels were compared between normal pregnant women and pregnant women with supplementation beneficial affect was seen and the differences were very highly significant. Although the catalase level was decreased in supplementation group rest of other parameters showed the positive outcome.

As soluble antioxidant levels are highly influenced by maternal dietary intake, dietary factors could play a role in preeclampsia. One study has suggested that an intake of vitamin C below the recommended dietary allowance may double the preeclampsia risk, but there is no evidence for a similar effect for vitamin E. In view of the known synergy between vitamin C and E, impairment of vitamin E regeneration because of the low plasma vitamin C concentrations could compromise the free radical scavenging capacity of vitamin E in women with preeclampsia (Chan, 1993).

Some antioxidants not only detoxify free radicals but also are involved in redox-sensitive gene expression, inhibition of apoptosis, and may have anti-inflammatory properties. Importantly Vit E may have relation to preeclampsia prevention, because vitamin E may not only inhibit the lipid peroxidation chain reaction but also could minimize the excessive generation of free radicals by inhibition of NAD(P)H oxidase in both placental tissue and maternal neutrophils reduce placental apoptosis, and inhibit leukocyte and endothelial cell activation (Raijmakers *et al.*, 2004).

 © International Journal of Research in Pharmaceutical Sciences

CONCLUSIONS

Our study has absorbed on the role of oxidative stress and antioxidant capacity in preeclampsia. By assessing markers of lipid peroxidation and antioxidant capacity, we achieved unequivocal evidence for oxidative stress in this disorder. In the light of the above interpretations it can be determined that pre-eclamptic pregnant women have higher levels of MDA and super oxidase dismutase while decreased level of reduced glutathione and catalase and the difference were very highly significant. Our finding on role of antioxidant in preeclampsia when compared with supplementation of Vitamins and normal pregnant women the result showed beneficial affect with the significant differences. It may provide new insight into a potentially modifiable way to prevent preeclampsia when these vitamins are administrated in diets.

Financial support and sponsorship

Nil

Conflicts of interest

Nil

REFERENCES

Ambad, D. R. S., Dhok, D. A. 2019. The Role Of Serum Urea, Creatinine, Uric Acid In Diagnosis Of Pre-Eclampsia And Eclampsia. *International Journal of Medical and Biomedical Studies*, 3(9).

Aouache, R., Biquard, L., Vaiman, D., Miralles, F. 2018. Oxidative Stress in Preeclampsia and Placental Diseases. *International Journal of Molecular Sciences*, 19(5):1496–1496.

Baliga, S., Chaudhary, M., Bhat, S., Bhatiya, P., Thosar, N., Bhansali, P. 2017. Determination of toral antioxidant capacity of saliva in sickle cell anemic patients – A cross-sectional study. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, 35(1):14–14.

Brown, M. A., Lindheimer, M. D., Swiet, M., De, Assche, A., Van, Moutquin, J. M. 2001. The Classification And Diagnosis Of The Hypertensive Disorders Of Pregnancy: Statement From The International Society For The Study Of Hypertension In Pregnancy (Isshp). *Hypertension in Pregnancy*, 20(1).

Buonocore, G., Perrone, S., Tataranno, M. L. 2010. Oxygen toxicity: chemistry and biology of reactive oxygen species. In Seminars in Fetal and Neonatal Medicine . *WB Saunders*, 15(4):186–190.

Chamy, V. M., Lepe, J., Álvaro Catalán, Retamal, D., Escobar, J. A., Madrid, E. M. 2006. Oxidative stress is closely related to clinical severity of pre-eclampsia. *Biological Research*, 39(2):229–236.

Chan, A. C. 1993. Partners in defense, vitamin E and vitamin C. *Canadian Journal of Physiology and Pharmacology*, 71(9):725–731.

Curtin, W. M., Weinstein, L. 1999. A Review of HELLP Syndrome. *Journal of Perinatology*, 19(2):138–143.

Faizal, P., Satheesan, B., Vinod, B., Augusti, K. T. 2017. Evaluation of Antioxidant, Lipid Peroxidation and Toxic Effects after Pomegranate Intake in Healthy Human Volunteers. *International Journal of Clinical Medicine*, 08(01):12–20.

Gaikwad, K. B., Joshi, N. G., Selkar, S. P. 2017. Study of nitrosative stress in 'Pregnancy Induced Hypertension. *Journal of clinical and diagnostic research: JCDR*, 11(3):6–08.

Gohil, J. T., Patel, P. K., Gupta, P. 2011. Evaluation of Oxidative Stress and Antioxidant Defence in Subjects of Preeclampsia. *The Journal of Obstetrics and Gynecology of India*, 61(6):638–640.

Góth, L. 1991. A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196(2-3):143–151.

Koracevic, D. 2001. Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology*, 54(5):356–361.

Llurba, E., Gratacós, E., Martín-Gallán, P., Cabero, L., Dominguez, C. 2004. A comprehensive study of oxidative stress and antioxidant status in preeclampsia and normal pregnancy. *Free Radical Biology and Medicine*, 37(4):557–570.

Maarten, T. M., Raijmakers, R., Dechend, L. P. 2020. Oxidative Stress and PreeclampsiaRationale for Antioxidant Clinical Trials. *Brief Reviews*.

Mccord, J. M., Fridovich, I. 1988. Superoxide dismutase: The first twenty years (1968–1988). *Free Radical Biology and Medicine*, 5(5-6):363–369.

Menon, K., S, G, V. 2007. Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with pregnancy induced hypertension. *Indian J Physiol Pharmacol*, 51(3):284–292.

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., Milner, A. 1993. A Novel Method for Measuring Antioxidant Capacity and its Application to Monitoring the Antioxidant Status in Premature Neonates. *Clinical Science*, 84(4):407–412.

Patil, S. B., Kodliwadmath, M. V., Kodliwadmath, S. M. 2007. Role of lipid peroxidation and enzymatic antioxidants in pregnancy-induced hypertension. *Clinical and experimental obstetrics & gynecology*, 34(4):239–241.

Pompella, A., Visvikis, A., Paolicchi, A., De Tata, V., Casini, A. F. 2003. The changing faces of glutathione, a cellular protagonist. *Biochemical Pharmacology*, 66(8):1499–1503.

Poston, L., Igosheva, N., Mistry, H. D., Seed, P. T., Shennan, A. H., Rana, S., Karumanchi, S. A., Chappell, L. C. 2011. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. *The American Journal of Clinical Nutrition*, 94(suppl_6):1980S–1985S.

Raijmakers, M. T. M., Dechend, R., Poston, L. 2004. Oxidative Stress and Preeclampsia. *Hypertension*, 44(4):374–380.

Wasnik, R. R. 2017. Evaluation of Serum Zinc and Antioxidant Vitamins in Adolescent Homozygous Sickle Cell Patients in Wardha, District of Central India. *Journal Of Clinical And Diagnostic Research*, 11(8):1–03.

Yadav, S., Agrawal, M., Hariharan, C., Dewani, D., Vadera, K., Krishna, N. 2018. A comparative study of serum lipid profile of women with preeclampsia and normotensive pregnancy. *Journal of Datta Meghe Institute of Medical Sciences University*, 13(2):83–83.