Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2015; 3(4B):1703-1706 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2015.v03i04.019

Research Article

Free Radicals and Antioxidants Enzymes Status in Normal Pregnant Women

Shrivastava Swati[1*], Kumar Sarvesh[2], Shrivastava Sourabh[3]
[1]Department of Biochemistry, G. R. Medical College, Gwalior, Madhya Pradesh
[2]Department of Community Medicine, RUHS College of Medical Sciences, Jaipur, Rajasthan
[3]Department of Anesthesiology, G. R. Medical College, Gwalior, Madhya Pradesh

***Corresponding author**
Swati Shrivastava,
Email: swatishrivastava547@gmail.com

Abstract: Pregnancy is associated with increased oxidative stress, and exaggeration of oxidative damage is considered responsible factor in pregnancy complications such as preeclampsia. There is, however, only little information regarding the longitudinal change of oxidative stress during pregnancy, so the main objectives of the study to find out the status of lipid peroxidation product (MDA) & antioxidants enzymes (like GPx and SOD) in pregnant vs. non- pregnant women.A cross sectional hospital based study, comprised of 30 normal pregnant women (third trimester) as cases and 30 healthy non pregnant women as controls. Malondialdehyde (MDA) and enzymatic antioxidant Glutathione peroxidase (GPx), Superoxide dismutase (SOD) levels were determined by using spectrophotometric methods. MDA and GPx levels were found to be highly significant (p value <0.001) higher in cases than controls. SOD was also found significantly (p value <0.01) higher when compared to controls. In normal pregnancies, there is an increase in free radical production and lipid peroxidation towards the end of pregnancy when compared with non-pregnant women. In a parallel fashion, total antioxidant capacity gradually increases during pregnancy, leading to oxidative balance maintained throughout pregnancy.
Keywords: Malondialdehyde, Oxidative stress, Glutathione peroxidase, Superoxide dismutase, Reactive oxygen species, Pregnancy

INTRODUCTION

Pregnancy refers to the fertilization and development of one or more offspring, known as a fetus or embryo, in a woman's uterus [1]. Pregnancy is a stressful condition associated with many physiological and metabolic alterations in functions to a considerable extent [2]. Normal pregnancy is associated with high metabolic demand and higher requirement for tissue oxygen resulting in increased oxidative stress and antioxidant defenses [3]. There is a complex interaction of the prooxidants and antioxidants that results in the maintenance of intracellular homeostasis. A state of oxidative stress is initiated due to the imbalance between the prooxidants and antioxidants [4]. Oxidative stress (OS) has found to affect multiple physiological processes, from oocyte maturation to fertilization, embryo development and pregnancy. OS plays a role during pregnancy and normal parturition and in initiation of pre-term labor [5]. Relentless formation of free radicals with the absence of proper antioxidant balance causes pathological changes in cells and tissue of female reproductive tract affecting functions like oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of fluid filled cavity, blastocyst, luteolysis, and luteal maintenance in pregnancy [6].

Aerobic metabolism is found to be associated with the generation of free radicals. Generation of free radicals is a normal physiological process. They act on lipids causing lipid peroxidation [7]. There are two major types of free radical species— Reactive oxygen species (ROS) and reactive nitrogen species (NOS). In a healthy person, ROS and antioxidants remain in balance. Disruption in this balance, towards an overabundance of ROS causes OS. In most cases, OS appears to be a result of increased generation of ROS, rather than a depletion of antioxidants [6]. Pregnancy, mostly because of the mitochondria-rich placenta, is a condition favouring oxidative stress [8]. Many complications in pregnancy and birth defects have found to be related to the oxidative stress, free radical damage. Cells have developed a wide range of antioxidants systems in order to limit the production of ROS, inactivate them and to repair the cell damage. Antioxidants play an essential role in the development and growth of the fetus, maintenance of a healthy pregnancy - and even before pregnancy, in fertility and conception [9].

Therefore, pregnancy is confronted with aggressive episodes of progressive and periodic changes in metabolic and physiological profile. Pregnancy while not a disease often accompanied by a high-energy demand of many bodily functions and an increased oxygen requirement. This triggered aerobic environment should primarily be responsible for raised oxidative stress in pregnancy.

METHODOLOGY

A cross sectional hospital based study, was carried out in the Department of Biochemistry, G. R. Medical College over a period of 6 months from 1st July 2011 to 31st December 2011. The study comprised of 30 healthy non pregnant women as controls and 30 normal pregnant women (third trimester) as subjects. The subjects and controls were in the range of 20 – 35 years. The sample size was calculated using the appropriate formula Z2 PQ/d2 where P & Q were taken as 0.5 to get the maximum sample size with 80% confidence interval with 10 per margin of error. The data was entered in MS Excel and analyzed using SPSS software. Standard statistical techniques were applied according to suitability of data. Significance of differences in various parameters between the two groups was analyzed for significance using the student's t-test.

Inclusion criteria

All the pregnant women, aged, 20 to 35 years, (irrespective of parity), who were in their third trimester were included- Pregnancies without any significant medical & obstetrical complications and pregnancies with single live fetus cases who are neither in spontaneous nor induced labor.

Exclusion criteria

To omit the cases from subject group, Teenage pregnancy or elderly primigravida, multiple pregnancies, Pregnancies with PIH, GDM Pregnancies with other medical disorders like renal disease, cardiac disease, anemia, jaundice etc.

Selection criteria for control group

All the non-pregnant women in their reproductive age group those without any significant medical complications. Blood samples of pregnant women were collected from antenatal ward of Kamla Raja Hospital, Obstetrics & Gynecology department of G.R. Medical College Gwalior (M.P.).

Blood samples were analyzed for following parameters

- Product of lipid peroxidation i.e. Malondialdehyde (MDA) by the method of Jean CD *et al*. [10]
- Glutathione peroxidase (GPx) by the method of Hafeman D.G. *et al*. [11]
- Superoxide dismutase (SOD) by the method of Mishra H. P. and Fridovich I [12]

RESULTS AND DISCUSSION

In our study the oxidative status during pregnancy was evaluated by analyzing pro-oxidant and antioxidant in blood. The RBCs were selected for the estimation of these enzymes because they are easily accessible. They are rich in thiol functions and are potentially involved in attack from and protection against free radicals [13]. Lipid peroxides i.e. PMDA is used as a marker for pro-oxidant while glutathione peroxidase and superoxide dismutase as antioxidants. In normal pregnancies it has been observed that there is an increase in free radical production and lipoperoxidation towards the end of pregnancy when compared with non-pregnant women. Total antioxidant capacity gradually increases during pregnancy that leads to oxidative balance maintained throughout pregnancy [14].

This study shows that physiological state of pregnancy causes an increase in the amount of lipid peroxidation products in the blood. Arikan *et al*. [15] had reported the significant increase in the level of thiobarbituric acid during normal pregnancy. Lipid peroxides are formed when lipids interact with oxygen radicals. The human placenta produces lipid peroxides that are secreted mainly to the maternal side of the placenta [16] and markers of increased lipid peroxidation are observed during normal pregnancy [17].

In the present study, the plasma concentration of malondialdehyde (MDA) was found to be higher in pregnancy state than in non-pregnant state, and the concentration increases with progressing gestational age. This is in agreement with study by Patil *et al*. [18]. This could be due to increased generation of ROS because of increased oxygen demand during pregnancy [19]. MDA, a product of lipid peroxidation induced by ROS, is well correlated with the degree of lipid peroxidation [20, 21]. Our results revealed a highly significant ($p < 0.001$) increase in MDA content in pregnant women as comparison to non – pregnant women (As shown in Table 1)

In our study the erythrocyte antioxidant enzyme i.e. superoxide dismutase (SOD) ($p<0.01$) and glutathione peroxidase (GPx) ($p<.001$) activities have been increased significantly in normal pregnant women (as shown in table 1). SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GPX, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes [22]. The rise in the activity of GPX could be due to its induction to counter the effect of increased oxidative stress.

Table 1: Showing status of MDA, GPx and SOD in case and control group

Sl. No.	Variable	Mean ± SD		Confidence interval		t-Statistic	p-value
		Case (N=30)	Control (N=30)	Lower	upper		
1	Status of MDA (nmol/ml)	6.76± 3.25	2.77± 0.23	2.80	5.18	6.71	0.000
2	Status of GPx (Enzymes Units/mg% of Hb)	13.41±5.03	8.14 ± 2.80	3.18	7.38	5.02	0.000
3	Status of SOD (Enzymes unites/mg protein/ml)	15.86±6.09	11.73± 3.97	1.47	6.79	3.12	0.003

Several studies have indicated that the antioxidative defense system is modified during pregnancy. Wisdom *et al*. [23] had shown that the activity of the superoxide dismutase (SOD), is reduced in the blood of pregnant women. In addition, Walsh and Wang [24] reported a deficiency of glutathione peroxidase (GPx) during pregnancy. GPx is an important antioxidant enzyme present in virtually all tissues limiting the generation of lipid peroxides and utilizing glutathione as its cofactor to convert lipid peroxides into relatively harmless hydroxylated fatty acids, water and glutathione disulfide [25].

Xihua Chen *et al*. [26] demonstrated that normal pregnancy is associated with increased GPx activity and insulin resistance. There are ethnic differences in antioxidant response and dietary fat intake. Their findings suggested a potential link among antioxidant defenses, insulin resistance and dietary intake of fat.

The presence of micronutrients in diet is required to maintain the activity of antioxidant enzymes in erythrocytes like SOD activity is dependent on copper [27, 28]. Natural endogenous antioxidant enzymes activity is enhanced significantly in response to oxidative stress, to maintain the state of equilibrium in favour of antioxidant defence. A regular check of antioxidant enzymes can prevent maternal and early foetal damage.

Increased knowledge regarding the importance of the antioxidants and mechanism in maintaining successful pregnancy and determining both the long and short term health of both mother and baby are essential in order to make a key focus for future health strategies in improving pregnancy outcomes. It is particularly important in relation to pre-eclampsia and fetal growth restriction, where oxidative stress is an important component to etiology of these conditions [29].

Though pregnancy is culmination of maths of anticipation and expectation associated with excitement and apprehension in every woman’s life, its outcome may cover entire spectrum from glorious fulfillment to catastrophic loss both for mother and fetus.

CONCLUSION

Our study showed that during normal pregnancy lipoperoxidation is increased when compared with healthy, non-pregnant women. As total antioxidant capacity (enzymatic and non-enzymatic) increased, a compensatory balance of the injury/defense ratio was maintained. Therefore, oxidative equilibrium persists throughout pregnancy. Implications of maternal systemic oxidative stress in normal pregnancy and in pregnancy complicated by preeclampsia like disorders. Supplementation of micronutrient and vitamins is advisable to maintain the functional activity of antioxidant enzyme and to prevent oxidative stress in cell and its complications.

REFERENCES

1. Robinson, Victor; "Primipara". The Modern Home Physician, A New Encyclopedia of Medical Knowledge. WM. H. Wise & Company, New York, 1939.
2. Walsh SW; Lipid peroxidation in pregnancy. Hypertension in Pregnancy, 1994; 13(1): 1-32.
3. Knapen MFCM, Zusterzeel PLM, Peters WHM, Steegers EAP; Glutathione and glutathione – related enzymes in reproduction. Eur J Obstet Gynecol Repord Biol., 1999; 82(2): 171-184.
4. Gupta S, Sekhon L, Aziz N, Agarwal A; the impact of oxidative stress on female reproduction and art: an evidence-based review. In Makrigiannakis A, Rizk B, Velasco JG, Sallam G; Infertility and assisted reproduction, Newyork, Cambridge UP, 2008: 178-186.
5. Agarwal A, Gupta S, Sharma RK; Role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology, 2005, 3: 28.
6. Agarwal A, Gupta S; The role of free radicals and antioxidants in female infertility and assisted reproduction. US Genito-Urinary Disease, 2006: 60-65.
7. Cheesman KH, Slater TF; An introduction to free radical biochemistry. Br Med Bull., 1993; 49(3): 481-493.
8. Casanueva E, Viteri FE; Iron and oxidative stress in pregnancy. J Nutr., 2003; 133(5 Suppl 2):1700S-1708S.

9. Polak G, Kozioł-Montewka M, Gogacz M, Błaszkowska I, Kotarski J; Total antioxidant status of peritoneal fluid in infertile women. Eur J Obstet Gynecol Reprod Biol., 2001; 94(2): 261-263.
10. Jean CD, Maryse T, Marie JF; Plasma malondialdehyde levels during myocardial infarction. Clin Chim Acta, 1983; 129(3): 319–322.
11. Hafeman DG, Sunde RA, Hoekstra W; Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr., 1974: 104(5): 580-587.
12. Misra HP, Fridovich I; The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem., 1972; 247: 3170-3175.
13. Palier MS, Hoidal JR, Ferris FT; Oxygen free radicals in ischemic acute renal failure in rats. J Clin Invest., 1984; 74(4): 1156–1164.
14. Toescu V, Nuttall SL, Martin U, Kendall MJ, Dunne F; Oxidative stress and normal pregnancy. Clin Endocrinol., 2002; 57(5): 609-613.
15. Arikan S, Konukoglu D, Arikan C, Akcay T, Davas I; Lipid peroxidation and antioxidant status in maternal and cord blood. Gynecol Obstet Invest., 2001; 51(3): 145- 149.
16. Walsh SW, Wang Y; Deficient glutathione peroxidase activity in preeclampsia is associated with increased placental production of thromboxane and lipid peroxides. American Journal of Obstetrics and Gynecology, 1993; 169(6): 1456-1461.
17. Morris JM, Gopaul NK, Eachresen MJ, Knight M, Linton EA, Dhur S Anggard EE *et al*.; Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. British Journal of Obstetrics and Gyneacology, 1998; 105(11): 1195-1199.
18. Patil SB, Kodliwadmath MV, Kodliwadmath SM; Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. J Obstet Gynecol India, 2006; 56(5): 399-401.
19. Halliwell B, Gutteridge JM; Role of free radicals and catalytic metal ions in human disease. An overview. Methods Enzymol., 1990; 186: 1-85.
20. Suhail M, Faizul-Suhail M, Hina K; Alterations in antioxidant and pro-oxidant balance in preeclampsia. Impact on erythrocyte osmotic fragility. Biochemia Medica, 2008; 18(3): 331–341.
21. Vanderlelie J, Venardos K, Clifton VL, Gude NM, Clarke FM, Perkins AV; Increased biological oxidation and reduced anti-oxidant enzyme activity in pre-eclamptic placentae. Placenta, 2005; 26(1): 53-58.
22. Chandra R, Aneja R, Rewal C, Konduri R, Dass K, Agarwal S; An opium alkaloidpapaverine ameliorates ethanol induced hepatotoxicity: diminution of oxidative stress", Indan Journal of Clinical Biochemistry, 2000; 15(2): 155-160.
23. Wisdom SJ, Wilson R, Mckillop JH, Walker JJ; Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. American Journal of Obstetrics and Gynecology, 1991; 6(6 Pt 1): 1701-1705.
24. Walsh SW, Wang Y; Secretion of lipid peroxides by the human placenta. American Journal of Obstetrics and Gynecology, 1993; 169(6): 1462-1466.
25. Levander OA; Selenium and sulfur in antioxidant protective systems: relationships with vitamin E and malaria. Proceedings of the Society of Experimental Biology and Medicine, 1992; 200(2): 255-259.
26. Chen X, Scholl TO, Leskiw MJ, Donaldson MR, Stein TP; Association of glutathione peroxidase activity with insulin resistance and dietary fat intake during normal pregnancy. The Journal of Clinical Endocrinology & Metabolism, 2003; 88(12): 5963–5968.
27. Bertger WJ, Fish TJ, O'dell BL; Effects of dietary copper and zinc erythrocyte stability and superoxide dismutase activity. Proceedings of the Society for Experimental Biology and Medicine, 19798; 158: 279-282.
28. Wasowics W, Kantorski J, Perek D; Concentration of zinc and zinccopper superoxide dismutase activity in red blood cells in normal and children with cancer. Journal of clinical chemistry and clinical biochemistry, 1989; 27: 413-418.
29. Mistry HD, Williams PJ; The importance of antioxidant micronutrients in pregnancy. Oxid Med Cell Longev., 2011; 2011: 841749.