International Journal of TROPICAL DISEASE & Health
3(3): 224-232, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Product of Free Radical Injury and Antioxidant Status of Newly Diagnosed HIV Sero-positive Pregnant Women Seen in Osogbo, Nigeria

Adeniran Samuel Atiba[1], Patric Temi Adegun[2], Daniel Adebode Adekanle[3*], Babatunde Moses Duduyemi[4] and Rasaq Akintunde Akindele[3]

[1]Department Chemical Pathology, Ekiti State University, Ado-Ekiti, Nigeria.
[2]Department of Surgery, Urology Division, Ekiti State University, Ado-Ekiti, Nigeria.
[3]Department of Obstetrics and Gynaecology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria.
[4]Department of Anatomic Pathology, Ekiti State University Teaching Hospital, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ASA designed the study, wrote the protocol, performed the laboratory analysis of measured biochemical parameters and wrote the first draft of the manuscript. Author DAA performed the statistical analysis, and revised the final draft of the manuscript. Authors PTA and BMD managed the analyses of the study. Author RAA managed the literature searches. Furthermore, all authors read and approved the final manuscript.

Research Article

Received 20th March 2013
Accepted 16th May 2013
Published 15th June 2013

ABSTRACT

Aim: The presence of HIV and pregnancy is a double insult on the health of mothers. Both of these have been associated with free radical injury. The aim was to determine levels of malondialdehyde and antioxidants of newly diagnosed HIV sero-positive pregnant women.
Place and Duration of the Study: This study was carried out in pregnancy booking clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria. The study was carried out between June and December in 2011.

**Corresponding author: Email: adekanle2000@yahoo.co.uk;*

Methodology: Thirty nine HIV sero-positives pregnant, 58 HIV sero-negative pregnant and 56 HIV sero-negative non pregnant women were recruited into the study. Malondialdehyde, antioxidant enzymes (glutathione peroxidase and superoxide dismutase) and antioxidants vitamins (vitamins C and E) were analyzed accordingly from plasma and haemolysate prepared from subjects and controls.
Results: Highest and lowest plasma levels of malondialdehyde/µmol/l were found in HIV sero-positive pregnant women (5.93±0.57) and HIV sero-negative non pregnant women (0.95±0.62) respectively. Lowest levels of all the antioxidants were observed in HIV sero-positive pregnant women while highest level was found in HIV sero-negative non pregnant women. However, the differences were only statistically significant with Vitamin E and glutathione peroxidase when compared ($p<0.05$).
Conclusion: We conclude that Human immunodeficiency virus sero-positive pregnant women experience more free radical injury than those with HIV sero-negative pregnant women.

Keywords: Free radical; antioxidant; oxidative stress; human immunodeficiency virus; malondialdehyde.

1. INTRODUCTION

Human immunodeficiency virus (HIV), since its discovery, is still a great challenge on the health care delivery system worldwide. Several attempts have been made to find a cure but none has been able to see the light of the day. The presence of this virus in the body system poses a stress on body metabolism. Free radicals are generated and they affect cellular organelles, this may worsen the damage HIV has on the body system. Pregnancy also is a stressful physiological condition. This has been associated with an increased free radical injury [1] and lowered antioxidant status [1]. Furthermore, the presence of HIV and pregnancy may cause worse oxidative stress injury, that is, imbalance in oxidant and antioxidant in excess of oxidant.

Oxidative stress is produced in biologic system when oxidants (free radicals) attack polyunsaturated membrane lipid to generate more free radical especially when antioxidants are not adequate. Other cellular organelles are not spared by free radicals; they also attack proteins through the processes called carbonylation [2] and nitrosylation [3]. The attack on membrane lipid produces malondialdehyde (MDA) in the course of the reactions [4]. The detection of malondialdehyde is currently and widely used as product of free radical injury on membrane lipid [4].

In order to maintain a physiological metabolism, the progressive increase of oxidants has to be counterbalance by a parallel increase of total antioxidant capacity. Two major enzymatic antioxidants superoxide dismutase (SOD) and glutathione peroxidase (GPx), were shown to be progressively increased during pregnancy [5]. Also levels of the non enzymatic antioxidant, α-tocopherol, which is tightly bound to β-lipoproteins, increase during pregnancy [6].

In view of morbidity and mortality associated with HIV in pregnancy, attempt is being made to prevent mother to child transmission as well as to improve standard of living of mothers. The presence of HIV and pregnancy is a double insult on the health of mothers. Both of these health challenges have been associated with oxidative stress injury [7-10]. Improvement in the antioxidant status and reduction in oxidant may improve the standard of

living of HIV sero-positive pregnant women. Beneficial effects of antioxidants have been demonstrated in clinical condition like dementia [11]. This study was therefore designed to determine the levels of antioxidant enzymes; glutathione peroxidise (GPx), superoxide dismutase (SOD) and vitamins C and E as well as product of free radical attack on membrane lipid, malondialdehyde (MDA).

2. MATERIALS AND METHODS

The study site was pregnancy booking clinic of Ladoke Akintola University of Technology Teaching Hospital. The study was carried out between June and December in 2011. Thirty nine HIV sero-positive pregnant women were recruited in the course of their routine HIV screening at booking. In the same way, 58 HIV sero- negative pregnant women were also recruited as controls. Furthermore, another 56 HIV sero-negative non pregnant women were recruited from general population. These people were recruited after getting their well informed consent. On recruitment, each subject was subjected to weight and height check as well as dip sticks urinalysis. Subjects with elevated blood pressure, suspected multiple gestations, those on any form of antioxidant vitamins and suspected gestational diabetes were all excluded from the study. All HIV sero-positive women were all newly diagnosed at booking.

About 10mls of venous blood was taken from all subjects and controls into lithium heparin specimen bottle. Both plasma and haemolysate were prepared. Blood collected was centrifuged at 3000g for 5 mins and supernatant plasma was extracted into plain specimen bottle. Haemolysate was prepared from red blood cell sediment inside lithium heparin specimen bottle. Red blood cells were washed three times with equal volume of normal saline solution and centrifuged at 3000g for 10minutes. Lysed erythrocytes were prepared by putting cells through three freeze-thaw cycles in dry ice and by the addition of four volumes of ice-cold distilled water. Cell membranes were removed by centrifugation, and the supernatant (haemolysate) was collected into a screw caped specimen bottles. Plasma and haemolysate were kept frozen at – 20ºC before analysis. Plasma was used for the laboratory analysis of MDA, GPx, Vit C and Vit E while haemolysate was used in the analysis of SOD. Human Immunodeficiency Virus (HIV 1 and 2) screening was done using dry chemistry kit manufactured by Alere Medical Co Limited, 357 Matsuhidal, Matsudo-shi, Chiba, 270-2214. Japan. Malondialdehyde was analysed using method of Satoh et al. [12], both SOD and GPx were analysed using ready to use commercially manufactured kits by Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim NT41 IQS, United Kingdom. Vitamins C and E were assayed using the Waters 616 HPLC machine manufactured by the Waters Corporation USA.

Data was entered and analysed using Statistical Package for the Social Sciences (SPSS) 16.0 putting level of significance at $p<0.05$. Variables were represented in mean and standard deviation and test of significant was done using one way ANOVA.

3. RESULTS

The mean age of women without pregnancy was 26.3±5.3 years and this is similar to the mean ages of pregnant women with (27.9±6.6 years) and without (27.9±4.3 years) HIV. The mean gestational age of HIV negative pregnant women was 24.0±1.3 weeks and that of HIV positive pregnant women was 24.3±2.8 weeks.

Table 1 shows mean ±SD of blood pressure and body mass index (BMI). There was no significant change (p=0.71) in systolic blood pressure (SBP) between HIV sero-negative non pregnant women (122.50±12.66 mmHg) and HIV sero-negative pregnant women (115.00±3.27 mmHg) when compared. Non pregnant HIV sero-negative (122.50±12.66 mmHg) and HIV sero-positive (127.00±7.26 mmHg) women when compared, showed no significant difference (p=0.61). Similarly, there was no significant changes in SBP (p=0.58) between SBP of HIV sero-negative (115.00±3.27 mmHg) and HIV sero-positive pregnant women (127.00±7.26 mmHg). Similarly findings were observed with diastolic blood pressure (DBP) when compared among the study groups. The diastolic blood pressure of HIV sero-negative non pregnant women was 69.23±8.37 mmHg, for HIV sero-negative pregnant women was 69.94±10.50 mmHg and for that of HIV sero-positive pregnant women was 80.72±7.90 mmHg. There was no also, significant changes (p=0.60;p=0.07) in BMI between HIV sero-negative non pregnant women (22.31±5.00 kg/m^2) and HIV sero-negative pregnant women (23.25±4.58 kg/m^2) as well as between HIV sero-negative non pregnant women (22.31±5.00 kg/m^2) and HIV sero-positive pregnant women (25.35±3.30 kg/m^2). Body mass index between HIV sero-negative pregnant women (23.25±4.58 kg/m^2) and HIV sero-positive pregnant women (25.35±3.30 kg/m^2) was also compared. There was no statistical difference; p=0.06.

Table 1. BMI, systolic and diastolic blood pressure of study groups

Variable	Comparison	Mean ±SD	*P*- Value
SBP	NonP Vs HNW	122.50±12.66 Vs 115.00±3.27	0.71
mmHg	NonP Vs HPW	122.50±12.66 Vs 127.00±7.26	0.61
	HNW Vs HPW	115.00±3.27 Vs 127.00±7.26	0.58
DBP	NonP Vs HNW	69.23±8.37 Vs 69.94±10.50	0.53
mmHg	NonP Vs HPW	69.23±8.37 Vs 80.72±7.90	0.47
	HNW Vs HPW	69.94±10.50 Vs 80.72±7.90	0.71
BMI	NonP Vs HNW	22.31±5.00 Vs 23.25±4.58	0.60
Kg/m^2	NonP Vs HPW	22.31±5.00 Vs 25.35±3.30	0.07
	HNW Vs HPW	23.25±4.58 Vs 25.35±3.30	0.06

Key: HNW-HIV seronegative pregnant women ** Statistically significant (p<0.05)*
HPW- HIV seropositive pregnant women
NonP- HIV seronegative Non pregnant women

Biochemical parameters were statistically compared and these are shown in Table 2. Plasma malondialdehyde was found to be lower in HIV sero-negative non pregnant women (0.95±0.62 µmol/l) when compared with HIV sero-negative pregnant women (4.73±0.64 µmol/l). This was statistically significant (p<0.001). Plasma MDA was also found to be lower in sero-negative non pregnant women (0.95±0.62 µmol/l) when compared with HIV sero-positive pregnant women (5.93±0.57). This was statistically significant (P<0.001). Plasma level of MDA was also found to be lower in HIV sero-negative pregnant women (4.73±0.64 µmol/l) than in HIV sero-positive pregnant women (5.93±0.57 µmol/l). This was however, not statistically significant (P=0.35). The plasma values of vitamin E (µmol/l) were found to be 31.35±13.79, 30.26±13.96 and 23.38±11.85 for HIV sero-negative non pregnant, HIV sero-negative and HIV sero-positive pregnant women respectively. Vitamin E was found to be higher in HIV sero-negative non pregnant women than HIV sero-negative pregnant women. However, this was not statistically significant;p=0.93. Plasma value of vitamin E was also found to be significantly (p<0.05) lower in HIV sero-positive pregnant women than in HIV sero-negative non pregnant women as well as HIV sero-negative pregnant women. The highest plasma level of vitamin C was found in HIV sero-negative non pregnant subjects

(43.41±26.93 µmol/l) while the lowest level was found in HIV sero-positive pregnant subjects (38.88±24.89 µmol/l). The plasma value of vitamin C in HIV sero-negative pregnant women was 41.37±22.02.µmol/l. However, these differences were not statistically significant (p>0.05). Glutathione peroxidase was found to be higher in HIV sero-negative non pregnant subjects (3253.98±2973.79 U/L) than in HIV sero-negative pregnant women (2956.22±1201.93 U/L). This was statistically significant, p<0.001. Plasma level of HIV sero-negative Non pregnant group (3253.98±2973.79 U/L) was also found to be higher than that of HIV sero-positive pregnant women (2696.27±1898.80 U/L). This was also statistical significant; p<0.001. Similarly, plasma level of GPx was found to be higher in HIV sero-negative pregnant women (2956.22±1201.93 U/L) than in the HIV sero-positive pregnant women (2696.27±1898.80 U/L). This was however, not statistically significant (p>0.05). The highest level of SOD was found in HIV sero-negative non pregnant women (98.59±45.32 U/ml) while the lowest was found in HIV sero-positive pregnant women (87.50±29.20 U/ml). The mean value for HIV sero-negative pregnant women was found to be 94.59±40.32 U/ml. However, when comparisons were done among the groups, there were no statistical significant differences; p>0.05.

Table 2. Comparison of mean ±SD of biochemical variables among the study groups

Variable	Comparison	Mean ±SD	*P*- Value
MDA	NonP Vs HNW	0.95±0.616 V 4.73±0.64	<0.001*
µmol/l	NonP Vs HPW	0.95±0.616 V 5.93±0.57	<0.001*
	HNW Vs HPW	4.73±0.64 V 5.93±0.57	0.34
Vit E	NonP Vs HNW	31.35±13.79 V 30.26±13.96	0.93
µmol/l	NonP Vs HPW	31.35±13.79 V 23.38±11.85	0.046*
	HNW Vs HPW	30.26±13.96 V 23.38±11.85	0.038*
Vit C	NonP Vs HNW	43.41±26.93 V 41.37±22.02	0.93
µmol/l	NonP Vs HPW	43.41±26.93 V 38.88±24.89	0.68
	HNW Vs HPW	41.37±22.02 V 38.88±24.89	0.91
GPx	NonP Vs HNW	3253.98±2973.79 V 2956.22±1201.93	<0.001*
U/L	NonP Vs HPW	3253.98±2973.79 V 2696.27±1898.80	<0.001*
	HNW Vs HPW	2956.22±1201.93 V 2696.27±1898.80	0.89
SOD	NonP Vs HNW	98.59±45.32 V 94.59±40.32	0.70
U/ml	NonP Vs HPW	98.59±45.32 V 87.50±29.20	0.89
	HNW Vs HPW	94.59±40.32 V 87.50±29.20	0.48

Key: HNW-HIV sero-negative pregnant women ** Statistically significant (p<0.05)*
HPW- HIV sero-positive pregnant women
NonP-HIV sero-negative Non pregnant women

4. DISCUSSION

The mean age and gestational age of the study groups were found to be similar. This has helped in an unbiased statistical comparison. Plasma malondialdehyde and antioxidants have been found to be affected as pregnancy advances [13-14]. Furthermore, the weight gain in the course of pregnancy also influences plasma malondialdehyde level [15]. The weight gain in pregnancy as well as other pathophysiology of pregnancy may be responsible for the oxidative stress found in them.

The presence of human immunodeficiency virus in biological system comes with a state of chronic inflammation. As a result of this there is an increased reactive oxygen species (ROS) which is an unstable free radical (oxidant). Furthermore, adaptive mechanism in pregnancy

may result to generation of ROS. This may be as a result of high oxygen demand resulting to hyperventilation seen in pregnant women. Reactive oxygen species attack some cellular organelles out which membrane polyunsaturated lipid is one. Cellular integrity is impaired with eventual cell death. In the course of these reactions, an intermediated product called malondialdehyde (MDA) is released and this is currently and widely being used as an index of free radical injury (lipid peroxidation) [4]. This may be so because of difficulty in measuring free radicals in the system probably because they are unstable. The effect of this free radical on membrane lipid may continue if they are not checked by some antioxidants. Antioxidant vitamins E and C as well as enzymes such as glutathione peroxidase and superoxide dismutase are known to counteract the ROS as well as reducing their damage. These antioxidants can be taken as supplements or consumed from the diet rich in them. Infact antioxidant like glutathione, apart from dietary consumption expected, N-acetylcycteine in the system influences its plasma increase. This has been shown to reduce oxidative stress in HIV infected patients [16].

Malondialdehyde as mentioned is a product of lipid peroxidation and it has been observed to be associated with the pathogenesis of some clinical conditions [17-19]. Our study observed plasma MDA to be higher in pregnant women who are HIV sero-positive when compared with those with HIV sero-negative pregnant women as well as HIV sero-negative non pregnant women. Suresh et al. [1] observed an increased MDA in HIV sero-positive adult population. This means that the generation of free radical is worse in HIV infected individuals. Reports supporting worsen free radical generation in HIV infected pregnant women are scarce in the literature. However, there are reports [20-21] to suggest that free radicals are generated in HIV sero-positive adult population. The addictive effect of these two free radical generated clinical conditions may support our finding. Free radical injury (oxidative stress) has been observed in women with normal pregnancy probably because pregnancy is a stressful physiological condition. However, Guptal et al. [22] in their systemic review observed that some studies reported no change in plasma MDA in normal pregnant women when compared with controls. This observation says that despite the insult from free radical attack from pregnancy, the plasma MDA remains the same. With this report, the addictive effect of pregnancy on free radical generation may be ignored. However, the HIV positive pregnant women may still have more free radical injury as also observed in our study. Never the less if subjects were recruited at a stage of HIV infection where free radical injury on membrane lipid has been appreciated, there may not be any difference in product of free radical injury. Also the antioxidant dietary constituent of the subjects may be helpful, that is overwhelmed free radical generation and its effects.

An antioxidant vitamin E is lipid soluble. It readily reacts with lipid radical produced in lipid membrane chain reaction to prevent membrane oxidation [23]. Vitamin C acts as a redox catalyst. It reduces and stabilizes reactive oxygen species such as hydrogen peroxide [24]. Both vitamins E and C have been shown to prevent free radical injury on membrane lipid. Our study observed a lower value of vitamins E and C in HIV sero-positive pregnant women when compared with HIV sero-negative pregnant women and sero-negative non pregnant women. Plasma vitamin E and C have been observed to be lower in pregnant woman [25] but reports on HIV sero-positive pregnant women was scarce in the literature. However, there are reports to suggest lower levels of vitamins E and C in HIV sero-positive adult [26]. The increased free radical injury observed with both pregnancy and HIV infection might have overwhelmed the available antioxidant vitamins. It may also be that the dietary composition of subjects was deficient in antioxidant vitamins measured and they were yet to be placed on antioxidant vitamins.

Antioxidant enzymes are also efficient antioxidant in counteracting the effect of free radical such as reactive oxygen species. Glutathione peroxidase protects cells from oxidative damage. It reduces lipid hydroperoxide to hydrogen peroxide and finally to water. Superoxide dismutase is another antioxidant vitamin that prevent reactive oxygen species form its deleterious effect on cell membrane. It catalyses the dismutation of reactive oxygen species. Superoxide is converted into the harmless oxygen and hydrogen peroxide. It should be expected that when there is increased free radical injury, antioxidants come to help. When these available antioxidants are consumed and they are not replaced, the lower levels of antioxidant are found at that point in time. Our study observed a reduced level of glutathione peroxidase and superoxide dismutase in HIV sero-positive pregnant women when compared with HIV sero-negative pregnant women as well as HIV sero-negative non pregnant women. There are reports to suggest decreased levels of SOD and Glutathione peroxidase in pregnancy [27-28] but not in HIV sero-positive pregnant women.

5. CONCLUSION

Human immunodeficiency virus sero-positive pregnant women experience more free radical injury than those with HIV negative pregnant women. Human immunodeficiency virus may pose more oxidative stress on pregnant women seen in Osogbo, Nigeria.

CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical clearance was obtained from the Ethical Committee of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria.

ACKNOWLEDGEMENTS

We recognise the support we got in subjects recruitment from nurses working in pregnancy booking clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Suresh DR, Vamseedher A, Pratibha K, Maruti PBV. Total antioxidant capacity- a novel early bio-chemical marker of oxidative stress in HIV infected individuals. Journal of Biomedical Science. 2009;16:61.
2. Frohnert BI, Bernlohr DA. Protein carbonylation, mitochondrial dysfunction, and insulin resistance. Adv Nutr. 2013;4(2):157-63.
3. Belén B, Antonia O, Emilio C, Salvador M. Oxidative stress and S-nitrosylation of proteins in cells. Br J Pharmacol. 2000;129(5):953–960.
4. Del MR. An approach to free radicals in medicine and biology. Acta Physiol. Scand. 1980;153–168.

5. Zusterzeel PL, Rutten H, Peters WH, Roelof HM, Steegers EA. Protein carbonyls in decidaus and placenta of preeclampsia women as markers for oxidative stress. Placenta. 2001;22:213-219.
6. Steinberg D, Parthasarathy S, Carew TE. Beyond Cholesterol: modifications of low-density hipoprotein that increase its autherogennivity. N Eng I Med. 1980;320:915–24.
7. Trotti R, Rondanelli M, anesi A, Gabanti E, Brustia R, Minoli L. Increased erythrocyte glutathione peroxidase activity and serum tumour necrosis factor-alpha in HIV-infected patients: relationship to on-going prothrombotic State. J Hematother Staem Cell. Res. 2002;11:369-75.
8. Luqman A, Salihu MA, Gafar A, Aremu IT, Soladoye AO. Effect of vitamin C on malondialdehyde in pregnant Nigerian women. Journal of Basic and Applied Sciences. 2008;4(2):105-108.
9. Hassan GI, Onu AB. Total vitamin C concentration in pregnant women: implications for a healthy pregnancy.Rev Bras Saude Matern Infant. 2006;6:293-296.
10. Ilhan N, Ilhan N, Simsek M. The changes of trace elements malonaldehyde levels and superoxide dimuthase activities in pregnancy with or without pre-eclampsia. Clin Biochem. Tul. 2002;35(5):393-7.
11. Steiner J, Haughey N, Li W, Venkatesan A, Anderson C, Reid R, et al. Oxidative stress and therapeutic approaches in HIV dementia. Antioxid Redox Signa. 2006;8(11-12):2089-100.
12. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 1978; 90: 37-43.
13. Basbug M, Demir I, Sernil S. Maternal erythrocyte malondialdehyde level inpre-eclampsia prediction: a longitudinal study. J. Perinat Med. 2003;31(6):469-74.
14. Sahu S, Abraham R, Vedavalli R, Daniel M. Study of lipid profile, lipid peroxidation and vitamin E in pregnancy induced hypertension. Indian J. Physiol Pharmacol. 2009;53(4):365-369
15. Violeta O, Ina G, Zose K, Danute K. Analysis of the level of free radical lipid peroxidation and antioxidative system activity during different pregnancy weight gain and multifetal pregnancy. Acta medica Lituanica. 2005;12(2):8-13.
16. Borges-Santos MD, Moreto F, Pereira PC, Ming-Yu Y, Burini RC. Plasma glutathione of HIV^+ patients responded positively and differently to dietary supplementation with cysteine or glutamine. Nutrition. 2012;28(7-8):753-6.
17. Ighodaro OM, Omole JO, Uwaifo AO. Effects of Chronic ethanol administration on body weight, reduced glutathione, malondialdeyde levels and glutathione-s-transferase activity in Rats. New York Science Journal. 2010;3(4):39-47.
18. Nnodin J, Anyadoh SO, Nwosu NEC. The antioxidant status and lipid peroxidation product of newly diagnosed and 6 weeks follow-up patients with pulmonary tuberculosis in Owerri, Imo State, Nigeria. Asian Pacific Journal of Tropical Diseases. 2011;292-294.
19. Qazi N, Neeru B, Imran M, Sanchit W, Harman K, Sheikh I. Malondialdehyde (MDA) and superoxide dismutase levels-distinguishing parameters between benign and malignant pleural effusions. Free Radicals and Antioxidants. 2012;2(2):8-11.
20. Batterham M, Gold J, Naidoo D, Lux O, Sadler S, Bridle S, et al. A prelimary open label dose comparison using an antioxidant regimen to determine the effect on viral load and oxidative stress in men with HIV/AIDS. Eur. J Clin Nutr. 2001;55:107-14.
21. Charles BS, Grace SM, Steven DD, Laurie AK, Craig MM. Glutathione, glutathione peroxidase and selenium status in HIV positive and HIV negative adolescents and young adults. Am J Clin Nutr. 2007;85:1173-181.

22. Guptal SA, Sekhon L, Agarwal R, Nabil A, Rishi RS, Agarwal A. Lipid Peroxidation and Antioxidant status in preeclampsia: a systemic review. Obstetrical and Gynaecological Survey. 2009;64(11):750–759.
23. Herrera E, Barbas C. Vitamin E: Action, metabolism and perspectives. Journal of Physiology and Biochemistry. 2001;57(2):43-56.
24. Padayatty ST, Katz A, Wang Y, Eck P, Kwon O. Vitamin C as an antioxidant evaluation of its role in disease prevention. Journal of the American College of Nutrition. 2003;22(11):18-35.
25. Cikol RJ, Steeger-Theunissen R, Thomas C, Boo TM, Merkus HM, Steegers EA. Longitudinal vitamin and homocystiene levels in normal pregnancy. Br. J Nurte. 2001;85:49-58.
26. Pyykko K, Ahotupa M, Uotila J, Tuimala R, Aarnio PK. Lipid Peroxidation products selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. Eur J Obstet Gynecol Reprod Biol. 1991;42:95–100.
27. Akalin FA, Baltacioğlu E, Alver A, Karabulut E. Total antioxidant capacity and superoxide dismutase activity levels in serum and gingival crevicular fluid in pregnant women with chronic periodontitis. J Periodontol. 2009;80(3):457-67.
28. Mistry HD, Wilson V, Ramsay MM, Symonds ME, Broughton Pipkin F. Reduced selenium concentrations and glutathione peroxidase activity in preeclamptic pregnancies. Hypertension. 2008;52(5):881-8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=228&id=19&aid=1484