

PRODUCT OF FREE RADICAL INJURY AND ANTIOXIDANT STATUS OF NEWLY DIAGNOSED HIV SEROPOSITIVE PREGNANT WOMEN SEEN IN OSOGBO, NIGERIA

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35 **ABSTRACT**

36 **Aim:** The presence of HIV and pregnancy is a double insult on the health of mothers. Both of
37 these have been associated with free radical injury. The aim was to determine levels of
38 malondialdehyde and antioxidants of newly diagnosed HIV sero-positive pregnant women.

39 **Place and duration of the study:** This study was carried out in pregnancy booking clinic of
40 Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria. The study
41 was carried out between June and December in 2011

42 **Methodology:** Thirty nine HIV sero-positive pregnant, 58 HIV sero-negative pregnant and
43 56 HIV sero-negative non pregnant women were recruited into the study.
44 Malondialdehyde, antioxidant enzymes (glutathione peroxidase and superoxide dismutase)
45 and antioxidants vitamins (vitamins C and E) were analyzed accordingly from plasma and
46 haemolysate prepared from subjects and controls

47 **Results:** Highest and lowest plasma levels of malondialdehyde/ $\mu\text{mol/l}$ were found in HIV
48 sero-positive pregnant women (5.93 ± 0.57) and HIV sero-negative non pregnant
49 women (0.95 ± 0.62). Lowest levels of all the antioxidants were observed in HIV sero-positive
50 pregnant women while highest level was found in HIV sero-negative non pregnant women.
51 However, the differences were only statistically significant with Vitamin E and glutathione
52 peroxidase when compared ($p < 0.05$).

53 **Conclusion:** We conclude that Human immunodeficiency virus sero-positive pregnant
54 women experience more free radical injury than those with HIV sero-negative pregnant
55 women.

56 **Key words:** Free radical, antioxidant, oxidative stress, Human immunodeficiency virus,
57 malondialdehyde.

58 **Abbreviation: Key:** *HNW-HIV seronegative pregnant women, NonP- HIV seronegative Non pregnant women,*
59 *HPW- HIV seropositive pregnant women, MDA-malonaldehyde, GPx-glutathione peroxidase, SOD- superoxide*
60 *dismutase*

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64 INTRODUCTION

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

74 Oxidative stress is produced in biologic system when oxidant (free radical) attack on
75 polyunsaturated membrane lipid generates more free radical without sufficient
76 antioxidants. The attack on membrane lipid produces malondialdehyde (MDA) in the
77 course of the reactions [2]. The detection of malondialdehyde is currently and widely used
78 as product of free radical injury on membrane lipid [2]

79 In view of morbidity and mortality associated with HIV in pregnancy, attempt is being made
80 to prevent mother to child transmission as well as to improve standard of living of mothers.
81 The presence of HIV and pregnancy is a double insult on the health of mothers. Both health
82 challenges have been associated with oxidative stress injury [3-6]. Improvement in the
83 antioxidant status and reduction in oxidant may improve the standard of living of HIV
84 seropositive pregnant women. This study was therefore designed to determine the levels of
85 antioxidant enzymes; glutathione peroxidase(GPx), superoxide dismutase(SOD) and
86 vitamins C and E as well as product of free radical attack on membrane lipid,
87 malondialdehyde (MDA)

88 MATERIALS AND METHODS

89 The study site was booking clinic of Ladoke Akintola University of Technology Teaching
90 Hospital. The study was carried out between June and December in 2011. Thirty nine HIV
91 sero-positive pregnant women were recruited in the course of their routine HIV screening
92 at booking. In the same way, 58 HIV sero- negative pregnant women were also recruited as
93 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. Ethical clearance was obtained from the ethical committee of Ladoke Akintola University of Technology Teaching Hospital, Osogbo

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 capped specimen bottles. Plasma and haemolysate were kept frozen at -20°C 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 [7], 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.

RESULTS

The mean age and gestational age of study groups are as shown in table 1. The mean age of women without pregnancy was 26.3 ± 5.3 and this is similar to the mean ages of pregnant women with (27.9 ± 6.6) and without (27.9 ± 4.3) HIV. The mean gestational age of HIV negative pregnant women was 24.0 ± 1.3 and that of HIV positive pregnant women was 24.3 ± 2.8 .

Table 2 shows mean $\pm$ 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) and HIV sero-negative pregnant women (115.00 ± 3.27) when compared. Non pregnant HIV sero-negative (122.50 ± 12.66) and HIV sero-positive (127.00 ± 7.26) women when compared, showed no significant difference ($p=0.61$). Similarly, there was no significant change ($p=0.58$) between SBP of HIV sero-negative (115.00 ± 3.27) and HIV sero-positive pregnant women (127.00 ± 7.26). 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 , for HIV sero-negative pregnant women was 69.94 ± 10.50 and for that of HIV sero-positive pregnant women was 80.72 ± 7.90 . 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) and HIV sero-negative pregnant women (23.25 ± 4.58) as well as between HIV sero-negative non pregnant women (22.31 ± 5.00) and HIV sero-positive pregnant women (25.35 ± 3.30). Body mass index between HIV sero-negative pregnant women (23.25 ± 4.58) and HIV sero-positive pregnant women (25.35 ± 3.30) was also compared. There was no statistical difference; $p=0.06$

Biochemical parameters were statistically compared and these are shown in table 3. Plasma malondialdehyde was found to be lower in HIV sero-negative non pregnant women (0.95 ± 0.62) when compared with HIV sero-negative pregnant women (4.73 ± 0.64). 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) 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) than in HIV sero-positive pregnant women (5.93 ± 0.57). This was however, not statistically

significant ($P=0.35$). The plasma values of vitamin E 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) while the lowest level was found in HIV sero-positive pregnant subjects (38.88 ± 24.89). The plasma value of vitamin C in HIV sero-negative pregnant women was 41.37 ± 22.02 . 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) than in HIV sero-negative pregnant women (2956.22 ± 1201.93). This was statistically significant, $p<0.001$. Plasma level of HIV sero-negative Non pregnant group (3253.98 ± 2973.79) was also found to be higher than that of HIV sero-positive pregnant women (2696.27 ± 1898.80). 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) than in the HIV sero-positive pregnant women (2696.27 ± 1898.80). 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) while the lowest was found in HIV sero-positive pregnant women (87.50 ± 29.20). The mean value for HIV sero-negative pregnant women was found to be 94.59 ± 40.32 . However, when comparisons were done among the groups, there were no statistical significant differences; $p>0.05$

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 [8-9]. Furthermore, the weight gain in the course of pregnancy also influences plasma malondialdehyde level [10]. 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)[2]. 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.

Malondialdehyde as mentioned is a product of lipid peroxidation and it has been observed to be associated with the pathogenesis of some clinical conditions [11-13]. 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 [14-15] to suggest that free radicals are generated in HIV sero-positive adult population. The additive 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, Gupta *et al* [16] in thier 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 additive 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 [17]. Vitamin C acts as a redox catalyst. It reduces and stabilizes reactive oxygen species such as hydrogen peroxide [18]. 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 [19] 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 [20]. 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 [21–22] but not in HIV sero-positive pregnant women.

248 CONCLUSION

249 Human immunodeficiency virus seropositive pregnant women experience more free radical
250 injury than those with HIV negative pregnant women. Human immunodeficiency virus may
251 pose more oxidative stress on pregnant women seen in Osogbo, Nigeria.

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254 booking clinic of Ladoke Akintola University of Technology Teaching Hospital, Osogbo. We
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340 Table 1: AGE AND GESTATIONAL AGE DISTRIBUTION OF SUBJECTS

	Non – Pregnancy	HIV–Ve pregnant women	HIV +Ve pregnant women
Mean Age (yr)	26.3±5.3	27.9±6.6	27.9±4.3
Mean Gestational Age		24.0±1.3	24.3±2.8

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358 Table 2:

359 BMI, SYSTOLIC AND DIASTOLIC BLOOD PRESSURE OF STUDY GROUPS

Variable	Comparison	Mean $\pm$ SD	P- Value
SBP mmHg	NonP Vs HNW	122.50 $\pm$ 12.66 Vs 115.00 $\pm$ 3.27	0.71
	NonP Vs HPW	122.50 $\pm$ 12.66 Vs 127.00 $\pm$ 7.26	0.61
	HNW Vs HPW	115.00 $\pm$ 3.27 Vs 127.00 $\pm$ 7.26	0.58
DBP mmHg	NonP Vs HNW	69.23 $\pm$ 8.37 Vs 69.94 $\pm$ 10.50	0.53
	NonP Vs HPW	69.23 $\pm$ 8.37 Vs 80.72 $\pm$ 7.90	0.47
	HNW Vs HPW	69.94 $\pm$ 10.50 Vs 80.72 $\pm$ 7.90	0.71
BMI Kg/m²	NonP Vs HNW	22.31 $\pm$ 5.00 Vs 23.25 $\pm$ 4.58	0.60
	NonP Vs HPW	22.31 $\pm$ 5.00 Vs 25.35 $\pm$ 3.30	0.07
	HNW Vs HPW	23.25 $\pm$ 4.58 Vs 25.35 $\pm$ 3.30	0.06

360 Key: HNW-HIV seronegative pregnant women

361 HPW- HIV seropositive pregnant women

362 NonP- HIV seronegative Non pregnant women

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375 Table 3: COMPARISON OF MEAN $\pm$ SD OF BIOCHEMICAL VARIABLES AMONG THE STUDY
376 GROUPS

Variable	Comparison	Mean $\pm$ SD	P- Value
MDA $\mu\text{mol/l}$	NonP Vs HNW	0.95 $\pm$ 0.616 V 4.73 $\pm$ 0.64	<0.001
	NonP Vs HPW	0.95 $\pm$ 0.616 V 5.93 $\pm$ 0.57	<0.001
	HNW Vs HPW	4.73 $\pm$ 0.64 V 5.93 $\pm$ 0.57	0.34
Vit E $\mu\text{mol/l}$	NonP Vs HNW	31.35 $\pm$ 13.79 V 30.26 $\pm$ 13.96	0.93
	NonP Vs HPW	31.35 $\pm$ 13.79 V 23.38 $\pm$ 11.85	<0.05
	HNW Vs HPW	30.26 $\pm$ 13.96 V 23.38 $\pm$ 11.85	<0.05
Vit C $\mu\text{mol/l}$	NonP Vs HNW	43.41 $\pm$ 26.93 V 41.37 $\pm$ 22.02	0.93
	NonP Vs HPW	43.41 $\pm$ 26.93 V 38.88 $\pm$ 24.89	0.68
	HNW Vs HPW	41.37 $\pm$ 22.02 V 38.88 $\pm$ 24.89	0.91
GPx U/L	NonP Vs HNW	3253.98 $\pm$ 2973.79 V 2956.22 $\pm$ 1201.93	<0.001
	NonP Vs HPW	3253.98 $\pm$ 2973.79 V 2696.27 $\pm$ 1898.80	<0.001
	HNW Vs HPW	2956.22 $\pm$ 1201.93 V 2696.27 $\pm$ 1898.80	0.89
SOD U/ml	NonP Vs HNW	98.59 $\pm$ 45.32 V 94.59 $\pm$ 40.32	0.704
	NonP Vs HPW	98.59 $\pm$ 45.32 V 87.50 $\pm$ 29.20	0.89
	HNW Vs HPW	94.59 $\pm$ 40.32 V 87.50 $\pm$ 29.20	0.48

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

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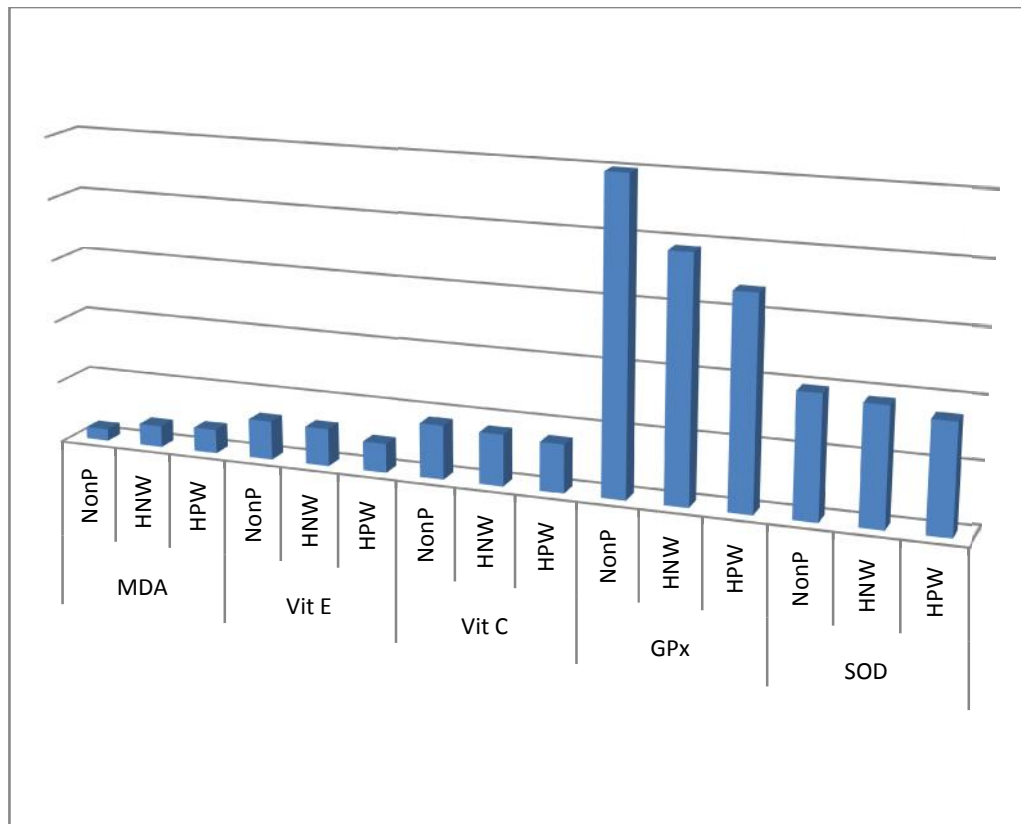
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389 Figure 1: TREND OF BIOCHEMICAL VARIABLES AMONG THE STUDY GROUPS



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391 Key: HNw-HIV sero-negative pregnant women
 392 HPw- HIV sero-positive pregnant women
 393 NonP-HIV sero-negative Non pregnant women
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* Statistically significant ($p < 0.05$)