Original Research Article

Haematological Parameters and Oxidative stress Changes in Apparently Healthy Pregnant Women in Bori, Nigeria

Abstract

Pregnancy is associated with alterations in haematological and oxidative stress parameters as physiological adjustments are made to accommodate the increasing demand from the fetus and the maintenance of maternal wellbeing. No study has evaluated the interaction between these parameters in a normal pregnant state. In this cross-sectional study, baseline values for haematological and oxidative stress changes were evaluated in pregnant women attending an ante-natal clinic at selected private clinics in Bori. A total of 100 subjects (80 pregnant women and 20 non-pregnant women) were recruited for the study. Haematological and oxidative stress parameters were determined following standard protocols. The result of the study shows a significant increase in the mean values of white blood cells, neutrophils, monocytes and catalase among the pregnant women compared to non-pregnant control while the mean value of the eosinophils was found to decrease among the pregnant women compared to the non-pregnant control ($p<0.05$). Neutrophils and catalase were significantly higher in the third trimester when compared with the non-pregnant control ($p<0.05$) A significant negative correlation was observed between catalase and mean cell volume; superoxide dismutase significantly correlated negatively with haemoglobin concentration, packed cell volume, ~~mean cell haemoglobin~~, mean cell haemoglobin concentration, total white blood cell and neutrophil counts. Glutathione significantly correlated negatively with neutrophils and monocytes while malondialdehyde significantly correlated with mean cell haemoglobin concentration, total white blood cell, monocytes and eosinophil and basophile counts. This study has demonstrated that there is a possible link between oxidative stress and haematological parameters among pregnant women.

Keywords: Haematology, pregnancy, oxidative stress, antioxidant enzymes

INTRODUCTION

Pregnancy describes the period of the conception and development of a fetus inside a woman's uterus usually lasting for about 40 weeks. This period is divided into 3 segments called trimesters of approximately 12 weeks or 3 months each [1, 2]. During this period, maternal physiology adapts considerably to accommodate the growing fetus. Hence, pregnancy is an anabolic state requiring several metabolic adjustments to support fetal growth demands and development while maintaining maternal homeostasis [3-5]. These changes include a considerable weight gain which puts the pregnant woman at a risk of dislocation [3, 6], changes in secretion of estrogen, progesterone, cortisol and growth hormone and a slight reduction in blood pressure [3, 7-9],

increase in respiratory rate and tidal volume and a reduction in functional residual capacity and peak expiratory flow rate [3, 10, 11].

Haematological changes occur in pregnancy as an adaptive tool in preparation for full fetal hematopoiesis and a cushion for expected blood loss in the course of delivery. Some of these haematological adaptations could appear pathological in non-pregnant states. For example, there is a general increase in plasma volume, red cell mass and adaptive immunological changes triggered by leukocytosis as white blood cells increase significantly with more increase in neutrophils stimulated by estrogen [3, 12-14]. These parameters under stable conditions are a reflection of the general health of pregnant women.

In the course of metabolism, living cells produce reactive oxygen (ROS) and reactive nitrogen species (RNS). ROS are molecular oxygen derivatives in form of hydroxyl radicals (OH), superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) which are characterized by an atom of oxygen with an unpaired electron and are continuously generated in all aerobic organisms in response to internal and external stimuli [15, 16]. On the other hand, Nitric oxide (NO) is an abundant intracellular messenger which regulates cardiovascular and neural physiology. Higher than normal levels of NO become deleterious under pathological conditions due to its high reactivity with other free radicals like the superoxide anions (O_2^-) to form peroxynitrite ($ONOO^-$), a strong oxidant capable of reacting and damaging biological molecules [17, 18]. These reactive species (ROS and RNS) have very high chemical reactivity. Their reactions result in peroxidation of lipids, enzymatic oxidation and extensive protein oxidation and degradation [19-21]. Antioxidants are molecules capable of limiting or inhibiting the oxidation of other molecules by scavenging ROS or inhibiting its production, hence preventing oxidative damage [22-24]. Oxidative stress results when there is a distorted balance between the generation of the reactive species and the scavenging ability of antioxidants [25, 26].

Oxidative stress results in varying effects on female reproductive function like ovarian steroid genesis, ovulation, implantation, oocyte maturation and luteal maintenance in pregnancy [27, 28] [25]. While this increase in metabolic rate during pregnancy ensures adequate fetal growth and development, it is associated with debilitating increased oxidative stress in the placental tissues, hence a need for an increased level of antioxidant enzymes to maintain the oxidative balance [29, 30]. Although ROS at physiological concentrations helps in the maintenance of pregnancy by stimulating cell proliferation and gene expression [31, 32], little data exist to suggest baseline information regarding oxidative stress levels and antioxidant capacity in uncomplicated pregnancies. The present study, therefore, is aimed at evaluating the pattern of haematological and oxidative stress changes in normal pregnancy to establish baseline values and possible relationships with haematological parameters during pregnancy.

MATERIALS AND METHODS

Study Population

This study was carried out in Bori, Rivers State, Nigeria. Bori remains the second-largest city in Rivers State after Port Harcourt and it is adjoined as the traditional headquarters of the Ogoni people. A predominately agrarian population with a land area of 20sqm ($50km^2$) and a population of two hundred and ninety-four thousand, two hundred and seventeen (294,217) people according to the population census of 2006 [33].

Research Design, Data and Sample Collection

This was a cross-sectional descriptive study. A total of 100 subjects (80 pregnant women and 20 non-pregnant women) were recruited from the study. The pregnant women were drawn from ante-natal clinics of selected private clinics in Bori, Rivers State. A simple questionnaire was used to obtain information such as the age and gestational age of the participants. About 3ml of blood was collected from the participants and dispensed into EDTA and plain sample bottles for the determination of haematological and oxidative stress parameters respectively.

Laboratory Analysis

Haematological parameters were assayed using an automated haematology analyzer (Mindray BC-2800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd) while oxidative stress parameters were analyzed using colorimetric procedures with standard kits (Elabscience, China)

Ethical Consideration

All procedures in this study were conducted following the highest ethical standards as contained in the World Medical Association (WMA) Helsinki Declaration of 1963 as amended in 2013 [34]. Request for consent form was signed by each prospective participant before recruitment into the study. The research design and protocol were approved by the Research Ethics Committee of the University of Port-Harcourt (UPH/CEREMAD/MM72/027).

Statistical Analysis

Data were analyzed using SPSS version 25. Social demographics were expressed in frequency and percentages. Student t-test was used to determine the differences in all the parameters between non-pregnant pregnant females while the ANOVA followed by an LDS posthoc analysis was used to determine the differences among the non-pregnant controls and the pregnant women in their various trimesters. A Pearson correlation was used to determine the correlation between haematological and oxidative stress parameters. All differences were considered significant at $p<0.05$.

RESULTS

Table 1: Demographic variables of the study population

Parameters		Non-Pregnant (n=20)	Pregnant (n=80)
Age Groups	<21	11 (55%)	10 (12.5%)
	21-30	6 (30%)	52 (65%)
	31-40	3 (15%)	18 (22.5%)
Marital Status	Single	18 (90%)	6 (7.5%)
	Married	2(1 0%)	74 (92.5%)

Table 1 below above shows the age and marital status of the study population. The result indicates that younger adults (<21years) participated in the study for the non-pregnant women while older adults (21-30years) took part in the study for the pregnant females. The non-pregnant women were predominantly single (90%) while most of the pregnant women were married (92.5%).

Parameters		Non-Pregnant (n=20)	Pregnant (n=80)
Age Groups	<21	11 (55%)	10 (12.5%)
	21-30	6 (30%)	52 (65%)
	31-40	3 (15%)	18 (22.5%)
Marital Status	Single	18 (90%)	6 (7.5%)
	Married	2(1 0%)	74 (92.5%)

Table 2: Hematological profile of pregnant and non-pregnant subjects in Bori, Rivers State.

Parameters	Non-Pregnant Control n=20	Pregnant Subjects n=80	t-test (p-value)
RBC ($x10^{12}$/L)	4.80±0.70	4.51±0.711	0.79
HB (g/dL)	13.10±1.86	12.24±2.22	0.22
PCV (%)	41.95±3.75	40.83±4.44	0.18
MCV (fL)	88.06±7.10	90.93±9.36	0.27
MCH (Pg)	27.66±4.79	27.22±4.40	0.17
MCHC (g/dL)	31.35±4.20	29.97±4.13	0.79
WBC($x10^9$/L)	2.90±2.31	4.11*±3.20	0.04
Neutrophils ($x10^9$/L)	1.30±0.92	2.24*±1.85	0.01
Lymphocytes ($x10^9$/L)	1.20±1.01	1.25±0.96	1.00
Monocytes ($x10^9$/L)	0.25±0.33	0.28*±0.27	0.01
Eosinophils ($x10^9$/L)	0.45±0.45	0.22*±0.34	0.01
Basophils ($x10^9$/L)	0.02±0.03	0.03±0.08	0.86
Platelets ($x10^9$/L)	158.00±60.77	137.28±52.58	0.39

Data are expressed as mean±standard deviation
*Significantly different compared to non-pregnant control ($p<0.05$)

Table 2 shows the mean values of some haematological variables [Red blood cell count (RBC), haemoglobin concentration (HB), packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration(MCHC), total white blood cell (WBC), neutrophil, lymphocytes, monocytes, eosinophil, basophil and platelet counts] of pregnant and non-pregnant subjects in Bori, Rivers State. The result reveals that mean values for WBC, neutrophils and monocytes were found to be significantly higher in the pregnant women compared to the non-pregnant control ($p<0.05$). Also, the mean value for eosinophils was found to be significantly lower in the pregnant subjects compared to the non-pregnant control ($p<0.05$)

Table 3: Haematological profile of non-pregnant and pregnant subjects in different trimesters

Parameters	Non-Pregnant (n=20)	First Trimester (n=8)	Second Trimester (n=40)	Third Trimester (n=32)	ANOVA (p-value)
RBC ($x10^{12}$/L)	4.80±0.70	4.25±0.89	4.48±0.72	4.63±0.66	0.21
HB (g/dL)	13.10±1.86	11.88±1.25	12.10±2.63	12.50±1.85	0.34
PCV (%)	41.95±3.75	39.75±4.74	40.50±4.83	41.50±3.87	0.47
MCV (fL)	88.01±7.10	93.16±10.05	90.83±9.48	90.51±9.27	0.54
MCH (Pg)	27.66±4.79	28.38±3.21	27.06±5.29	27.13±3.37	0.86
MCHC (g/dL)	31.35±4.20	30.53±1.99	29.78±5.08	30.06±3.17	0.58
WBC($x10^9$/L)	2.90±2.31	2.50±1.93	4.08±3.12	4.56±3.49	0.15
Neutrophils ($x10^9$/L)	1.30±0.92	1.25±0.04	2.18±1.58	2.56*±2.23	0.04
Lymphocytes ($x10^9$/L)	1.20±1.01	1.00±0.93	1.33±1.02	1.22±0.91	0.84
Monocytes ($x10^9$/L)	0.25±0.33	0.17±0.08	0.28±0.28	0.30±0.29	0.70
Eosinophils ($x10^9$/L)	0.45±0.45	0.12±0.09	0.23±0.33	0.24±0.40	0.08
Basophils ($x10^9$/L)	0.02±0.03	0.01±0.01	0.03±0.09	0.04±0.10	0.68
Platelets ($x10^9$/L)	158.00±60.77	128.88±22.45	131.40±41.09	146.72±12.06	0.27

Results are given as mean±S.D (Range)
*Significantly different compared to non-pregnant control $p<0.05$.

Table shows the haematological profile of the non-pregnant and pregnant subjects in different trimesters. The result shows a gradual rise in the mean values of RBC, HB and WBC from the first to the third trimesters. However, this slight increase was not significant when compared with the non-pregnant control ($P>0.05$). The result also indicate that mean value of neutrophils was found to be significantly higher in the third trimester when compared with the non-pregnant control ($p<0.05$).

Table 4: Oxidative stress parameters of pregnant and non-pregnant women in Bori, Rivers state

Parameters	Non-Pregnant Control n=20	Pregnant Subjects n=80	t-test (p-value)
Catalase (U/ml)	18.44±20.96	62.28*±44.11	0.01
Superoxide Dismutase (U/ml)	27.04±24.89	24.09±21.07	0.43
Malondialdehyde (µmol/L)	47.84±23.48	50.00±23.82	0.65
Glutathione (µmol/L)	1.36±0.34	1.50±0.54	0.01

Data are expressed as mean±standard deviation
*Significantly different compared to non-pregnant control ($p<0.05$)

Table 4 shows the mean values of some oxidative stress parameters [catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH)] of pregnant and non-pregnant subjects in Bori, Rivers State. The result shows that the mean value of Catalase (CAT) and GSH were significantly higher in the pregnant subjects in comparison to the control group ($p<0.05$).

Table 5: Oxidative stress parameters of non-pregnant and pregnant subjects in different trimesters

Parameters	Non-Pregnant (n=20)	First Trimester (n=8)	Second Trimester (n=40)	Third Trimester (n=32)	ANOVA (p value)
Catalase (U/ml)	18.44±20.96	59.06*±35.84	59.30*±37.23	66.80*±53.76	0.01
Superoxide Dismutase (U/ml)	27.04±24.89	29.53±24.25	20.97±19.34	26.62±22.39	0.57
Malondialdehyde (µmol/L)	47.84±23.48	48.72±16.70	49.24±24.45	51.27±25.05	0.96
Glutathione (µmol/L)	1.36±0.34	1.65±0.46	1.58±0.52	1.35±0.58	0.12

*Significantly different compared to control.
Results are given as mean±S.D (Range)

Table 5 shows the results for the oxidative stress markers for the non-pregnant and pregnant women in their various trimesters. The result indicates that the mean value for the Catalase was significantly higher in all the trimesters compared to the non-pregnant control ($p<0.05$).

Table 6: Correlation coefficients (r) between of Haematological and oxidative stress parameters among pregnant women in Bori, Rivers State.

Haematological Parameters	**Oxidative Stress Parameters**			
	CAT(U/ml)	SOD (U/ml)	MDA (µmol/L)	GSH (µmol/L)
RBC ($x10^{12}$/L)	0.336	-0.315	0.341	-0.293
HB (g/dL)	-0.224	-0.520*	-0.001	0.152
PCV (%)	-0.417	-0.532*	0.324	0.214
MCV (fL)	-0.598*	-0.396	-0.339	0.319
MCH (Pg)	-0.389	-0.475*	-0.428	0.297
MCHC (g/dL)	0.268	-0.395*	0.339*	0.195
WBC($x10^9$/L)	-0.274	-0.474*	0.550*	-0.439
Neutrophils ($x10^9$/L)	-0.259	-0.516*	0.423	-0.480*
Lymphocytes ($x10^9$/L)	-0.243	-0.310	0.454	-0.055
Monocytes ($x10^9$/L)	0.396	0.455	0.536*	-0.491*
Eosinophils ($x10^9$/L)	-0.381	0.307	0.597*	-0.363
Basophils ($x10^9$/L)	-0.303	-0.184	0.509*	-0.455
Platelets ($x10^9$/L)	-0.290	0.338	-0.385	-0.232

*Significant Pearson correlation ($p<0.05$)

Table 6 shows the values of Pearson correlation coefficient (r) between haematological and oxidative stress parameters among pregnant women in Bori, Rivers State. There was a significant negative correlation between catalase and MCV ($r=0.598$, $p<0.05$). Significant negative correlation was also observed between SOD and HB (-0.224, $p<0.05$), PCV (-0.417, $p<0.05$), MCH ($r=-0.389$, $p<0.05$), MCHC ($r=-0.395$, $p<0.05$), WBC ($r=-0.474$, $p<0.05$) and neutrophils ($r=0.516$, $p<0.05$). Similarly, significant negative correlation between GSH and neutrophils ($r=0.480$, $p<0.05$) and monocytes ($r=0.491$, $p<0.05$). However, a significant positive correlation was observed between MDA and MCHC ($r=0.339$, $p<0.05$), WBC ($r=0.550$, $p<0.05$) monocytes ($r= 0.536$, $p<0.05$), eosinophils ($r=0.597$, $p<0.05$) and basophils ($r=0.509$, $p<0.05$).

DISCUSSION

Pregnancy is associated with haematological adaptations and disturbance in the antioxidant status of the mother due to increased metabolic demand from the developing fetus [35-38]. The present cross-sectional study evaluated the haematological and oxidative stress parameters among pregnant (80) and non-pregnant (20) women in Bori, Rivers State.

The result of the study shows a significant increase in the mean values of white blood cells, neutrophils and monocytes among the pregnant women compared to non-pregnant control while the mean value of the eosinophils was found to decrease among the pregnant women compared to the non-pregnant control ($p<0.05$). There was a gradual rise in the mean values of red blood cell count and haemoglobin concentration and white blood cell count from the first to the third trimesters. The mean value of neutrophils was found to be significantly higher in the third trimester when compared with the non-pregnant control ($p<0.05$). Assessment of haematological profile has remained a simple, easy and reliable means of evaluating the general health status and in the case of pregnancy, it becomes more important in understanding physiological changes to interpret any need for therapeutic interventions during pregnancy [14, 39]. The slight decrease in the mean value of RBC and HB has been attributed to the increased demand for iron and oxygen with advancing gestation as more iron and oxygen are required to meet the needs of the developing foetus [40, 41]. However, the slight gradual rise in the mean RBC and HB with gestational age could be due to the effect of estrogen and progesterone which are released by the placenta and causes the release of renin from the kidneys which in turn enhances erythropoiesis [40, 42]. These PCV and HB changes in pregnancy have been observed elsewhere [39-41, 43].

It was also observed that the mean values of the WBC, neutrophil and monocyte count among the pregnant women were increased compared to non-pregnant control ($p<0.05$). There was a gradual rise observed in these parameters from the first to the third trimesters. Pregnancy has long been associated with leukocytosis due to increased inflammatory response with the increase in the circulation of neutrophils observed in the second month of pregnancy [12, 37] and remaining elevated till term. These results as the body build the immunity of the fetus through immunomodulation, immune tolerance and immunosuppression in the presence of a strong antimicrobial immunity [38, 44]. Also, an increase in neutrophils may be due to a stress response as a result of the redistribution of white blood cells between the marginal and circulating pools. It has also been suggested that there is a decrease in neutrophil apoptosis, chemotaxis and phagocytic activity thereby increasing their number in circulation [45, 46]. It has also been reported that anxiety, pain, nausea and vomiting can cause leukocytosis in the absence of infection [42]. Similar reports showing increased WBC and neutrophil among pregnant women has been reported by previous studies [13, 14, 38-42]. The significant reduction in eosinophil observed in this study could be attributed to possible parasitic infection [47, 48].

The results of the present study show that the mean value of CAT and GSH was found to be significantly higher among pregnant subjects compared to non-pregnant control ($p<0.05$), however, only mean values for CAT exhibited a rise in gestational age with a peak of 66.80±53.76 obtained for the third trimester (Table 5). Catalase has been described as a tetrameric haemin enzyme which reacts efficiently with hydrogen peroxide (H_2O_2) to form water and molecular oxygen [49, 50]. Hence, catalase functions to increase tolerance stress and in the adaptive response of cells thereby maintaining oxygen levels in the face of chemical reduction or due to interaction with a toxin [51, 52]. Similarly, glutathione (GSH) catalyzes the reduction of hydroperoxides thereby protecting mammalian cells against oxidative damage, hence providing an essential antioxidative defense mechanism alongside CAT in reaction to lipids and other organic hydro peroxidases [49, 50, 53]. Hormones associated with pregnancy increase basic metabolism and oxygen consumption and the use of fatty acids as the primary source of energy. These metabolic changes in most maternal and retroplacental tissues induce significant alterations in lipid metabolism with attendant significant variations in oxidant/antioxidant status [35, 54, 55]. The observed rise in CAT and GSH levels therefore could be due to their ability to remove ROS to avoid oxidative cell injury and reduce the development of chronic diseases [35, 36]. However, Singh *et al*. [56] observed a decrease in CAT in pregnancy while Oghagbon *et al*. [57] observed a reduction in CAT and GPx in pregnancy.

The relationship between haematological and oxidative stress parameters in the study (Table 6) shows that CAT was significantly correlated negatively with MCV while SOD significantly correlated negatively with HB, PCV, MCH, MCHC, WBC and neutrophils. Also, GSH significantly correlated negatively with neutrophils and monocytes while MDA significantly correlated with MCHC, WBC, monocytes, eosinophils and basophils. MCV measures the average size and volume of RBC. It is useful in the determination of the aetiology of anaemia. The mean value of MCV obtained among pregnant women in this study neither show any significant difference when compared to the non-pregnant women nor any pattern with gestational age (Tables 2&3). However, the decreasing tendency of MCV while CAT is increasing and vice versa could be a pointer to possible increasing activity of CAT to prevent or reduce oxidative damage to red cells. Superoxide dismutase (SOD) is the antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to molecular oxygen in the cytoplasm, mitochondria and the extracellular matrix of cells. SOD destroys superoxide by successive oxidation and reduction of the transition metal ion at the active site, converting it to peroxide that can in turn be destroyed by catalase or GPX reactions [49, 50]. An increasing tendency of SOD with decreasing blood cell parameters and vice versa shows a possible link between SOD and haematopoiesis during pregnancy.

CONCLUSION

Pregnancy is associated with alterations in haematological and oxidative stress parameters as physiological adjustments are made to accommodate the increasing demand from the fetus and

the maintenance of maternal wellbeing. While haematopoiesis is enhanced to fulfil increased tissue demand, there is a constant adjustment between pro-oxidant and antioxidant agents to avoid materno-fetal complications. The present study has demonstrated there is a possible link between oxidative stress and haematological parameters among pregnant women and hence antioxidants could be used routinely to reduce or prevent haematological complications during pregnancy.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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