A COMPARATIVE STUDY OF PLASMA VITAMIN C LEVELS IN PRE-ECLAMPTIC AND NORMOTENSIVE PREGNANCIES AT THE LAGOS UNIVERSITY TEACHING HOSPITAL

*ODEIGAH C C, **ODEIGAH L, **OLAGUNJU FA, ***SULEIMAN ZA, ^ISSA FY, ^^ADESINA KT, ^^SAIDU R, ^^RAJI HA, ^^AYENI MO ^^JIMOH AAG

*DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,

LAGOS UNIVERSITY TEACHING HOSPITAL (LUTH).LAGOS

**DEPARTMENT OF FAMILY MEDICINE

UNIVERSITY OF ILORIN TEACHING HOSPITAL

***DEPARTMENT OF ANAESTHESIA

^DEPARTMENT OF COMMUNITY MEDICINE AND EPIDEMIOLOGY

^^DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

UNIVERSITY OF ILORIN/UNIVERSITY OF ILORIN TEACHING HOSPITAL

CORRESPONDENCE:

JIMOH AAG
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY
UNIVERSITY OF ILORIN/UNIVERSITY OF ILORIN TEACHING HOSPITAL

ABSTRACT

Background: Pre-eclampsia remains a major cause of maternal and perinatal morbidity and mortality in our environment. Research into methods of prevention of this disorder has been hampered by a poor understanding of the pathological mechanisms leading to pre-eclampsia. Recent studies suggest that oxidative stress (with reduced antioxidant defences) could lead to free radical mediated endothelial dysfunction in pre-eclampsia.

Objectives: To determine the plasma vitamin C levels in pre-eclamptic and normotensive pregnant patients at LUTH, and to compare the levels of plasma vitamin C in both groups of patients with a view to investigate the association between plasma vitamin C level and pre-eclampsia.

Method: A comparative study of plasma vitamin C levels in pre-eclamptic and normotensive pregnancies. Informed consent was obtained from 90 women admitted to the labour ward with singleton pregnancies between 28 and 40 weeks, who were divided into two groups: 30 pre-eclamptic patients and 60 normotensive patients (each pre-eclamptic patient was matched for parity with 2 normotensive patients). Venous blood was obtained from all the participants. Plasma vitamin C level was measured using High Performance Liquid Chromatography (HPLC). Data was analyzed using inferential statistical methods.

Results: The results showed that mean plasma vitamin C level in the pre-eclamptic women was 119.3 ± 23.7 μg/ml, while the mean plasma vitamin C level in the normotensive pregnant patients was 246.5 ± 45.0 μg/ml. The mean concentration of plasma vitamin C level was significantly lower in the pre-eclamptic patients than in the normotensive patients ($t=14.5$; $p<0.001$)

Conclusion: The study showed that pre-eclampsia is associated with decreased concentration of plasma vitamin C. There is the need for a local study to investigate the effect of vitamin C supplementation on pre-eclampsia.

INTRODUCTION:

Pre-eclampsia is a multisystemic disorder of unknown cause that is unique to human pregnancy.[1] Pre-eclampsia complicates about 2-8% of all pregnancies.[2] The incidence of pre-eclampsia is about 5-8% of pregnancies in the United States of America.[3] Studies in Benin city[4], Nigeria and in Kenya[5] revealed prevalence rates of 5.6% and 5.4% respectively.

Preeclampsia is a leading cause of maternal mortality globally. The World Health Organization estimates that, worldwide, over 100 000 women die from preeclampsia each year.[6] The condition has remained one of the leading causes of maternal death in the UK over recent decades.[7] In the USA, pre-eclampsia is the third leading cause of pregnancy related deaths (after haemorrhage and pulmonary embolism), accounting for an estimated 790 maternal deaths per 100,000 live births.[8] In Nigeria, severe pre-eclampsia/eclampsia was the leading cause of maternal deaths at Port-Harcourt[9] and Ilorin[10], accounting for 37.5% and 27.8% of all maternal deaths respectively.

Despite intense research, the pathophysiology of the disease remains elusive. Strong evidence suggests that generation of placental oxidative stress is a key event in the pathology of pre-eclampsia.[11-14] The placental oxidative stress is thought to induce the placenta to release a mixture of factors, including inflammatory cytokines, anti-angiogenic factors and apoptotic debris, which culminates in an enhanced maternal inflammatory response and endothelial dysfunction.[15] Either through oxidative stress or other vasoactive substances being released from the placenta, activation of the vascular endothelium occurs.[3]

Endothelial cell damage and an impairment of endothelial cell function are thought to play an important role in the pathophysiology of pre-eclampsia.[16] It has been suggested that free radicals are likely promoters of maternal vascular malfunction. The highly reactive primary products of lipid peroxidation, lipid hydroperoxides, are formed when free radicals attack polyunsaturated fatty

acids or cholesterol in membranes or lipoproteins.[17] Alternatively, they can be formed by cyclooxygenase or lipoxygenase.[11] Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes.[11,16,17] However, uncontrolled lipid peroxidation can result in cellular dysfunction and damage.[15]

Under normal conditions, a variety of antioxidant mechanisms control lipid peroxidation.

Cells are able to defend themselves against Reactive Oxygen Species (ROS) damage through the use of enzymes such as superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins. Small molecule antioxidants such as ascorbic acid, tocopherol, uric acid and glutathione also play important roles as antioxidants. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals.

Evidence suggests that an imbalance between lipid peroxidation and antioxidant defenses could lead to endothelial dysfunction and free radical mediated endothelial cell injury in pre-eclampsia.[16] Several studies have demonstrated deficiency in the various protective antioxidant systems or increased utilization of antioxidants in pre-eclampsia as compared with normal pregnancy.[16-20]

Antioxidants such as vitamin C (ascorbic acid) have the capacity to scavenge free radicals and they function as inhibitors of reactive oxygen species, thereby controlling lipid peroxidation. A number of studies have demonstrated lower plasma/serum levels of vitamin C in women with pre-eclampsia.[16-18,22-23]

However, if further studies were to provide sufficient evidence to support the theory that lower levels of vitamin C occur in women with pre-eclampsia, this could have a significant impact on the management of the condition and on antenatal care as a whole.

As a potent water soluble antioxidant, vitamin C acts in a unique position to scavenge aqeous reactive oxygen species before these destructive substances have a chance to damage the lipids.[21]

Indeed, a number of studies have shown significantly lower vitamin C levels in pre-eclamptic patients as compared to healthy patients.[16-18, 22-23] However, there have been no documented local study addressing this issue. If a similar association can be demonstrated in our patients, this can form the basis for carrying out interventional studies to assess the use of vitamin C supplementation for prevention of pre-eclampsia in high risk patients. The present study is therefore designed to investigate the plasma levels of vitamin C in pre-eclamptic and normotensive patients in LUTH.

AIM AND OBJECTIVES

To investigate the association between plasma vitamin C levels and pre-eclampsia.

Objectives:

1. To determine plasma vitamin C levels in pre-eclamptic patients in LUTH.
2. To determine plasma vitamin C levels in normotensive pregnant patients in LUTH.
3. To compare the levels of plasma vitamin C between pre-eclamptic patients and normotensive pregnant patients in LUTH.

METHODOLOGY

This was a cross-sectional comparative study. The study was conducted at the labour ward of the Lagos University Teaching Hospital (LUTH). The patients seen are the booked antenatal patients of the hospital and unbooked patients referred from various maternity homes, private and other government hospitals. The plasma level of vitamin C was done in collaboration with laboratory scientists at the Central Research Laboratory, College of Medicine, University of Lagos.

All pregnant women admitted in the labour ward were eligible for the study if they satisfied the following criteria:

1. Were between 28 and 40 weeks of gestation.
2. The index pregnancy was a singleton gestation.
3. Had not been diagnosed to be hypertensive outside pregnancy.
4. Had no documented hypertension prior to the 20^{th} week of the index gestation.
5. Had no documented proteinuria prior to the 20^{th} week of the index gestation.
6. Had no history of use of Aspirin, vitamin C supplementation or smoking cigarettes during the antepartum period.

7. Had no other medical disorders concurrent with pre-eclampsia (such as diabetes mellitus, sickle cell disease or chronic hypertension).
8. Had given informed written consent.

Selection of Pre-eclamptic patients

These were consecutive patients admitted into the labour ward who met the ISSHP criteria for diagnosis of pre-eclampsia[2,3]: namely hypertension (one diastolic blood pressure reading ≥110mmHg or two consecutive diastolic blood pressure readings ≥90mmHg done at least 4 hours apart) and significant proteinuria (≥2+ on reagent strip carried out on 2 consecutive occasions at least 4 hours apart). Patients also fulfilled the inclusion criteria stated above.
The blood pressure was measured with a mercury sphygomanometer using the right arm. The patient was in the semi-recumbent position (45^{o} head up). The arm was in a horizontal position at the level of the heart. The diastolic blood pressure was measured using Korotkoff 5.

Selection of Normotensive Patients

For every pre-eclamptic patient recruited, two normotensive patient who had been matched for parity were recruited from the same study population using the following guidelines:

1. Met the inclusion criteria stated above.
2. No documented hypertension or proteinuria during the antepartum period.
3. Not found to be hypertensive or proteinuric on evaluation in the labour ward.
4. Admitted into the labour ward on the same day as the pre-eclamptic patients (or not more than 24 hours later if there were not enough patients) .

Evaluation of Pre-eclamptic and Normotensive patients recruited:

The purpose of the study was explained to the patients and informed written consent was obtained. Using a proforma, the sociodemographic data, parity, last menstrual period, calculated estimated gestational age at delivery, and perinatal outcome were assessed. In patients diagnosed to be pre-eclamptic, features suggestive of severe pre-eclampsia were sought.

SAMPLE COLLECTION

Five (5) millilitres of a peripheral venous blood sample was obtained by venepuncture from the participants before delivery. The blood sample was transported in Lithium heparin bottles to the laboratory after labeling them with a serial number.

LABORATORY METHODS:

Plasma vitamin C was measured using High Performance Liquid Chromatography (HPLC) as described by Liam et al.[11]

Chemicals and materials:

Vitamin C (L-ascorbic acid, purity: 99%) was obtained from USP Rockville, MD. Orthophosphoric acid (BDH chemicals), HPLC grade Methanol (Merck KGOA, 54271 darmstadt, Germany) and HPLC grade Acetonitrile (VWR int Ltd. Poole, BH15 ITD, Endland) were also used. Analytical grade EDTA was obtained from Kira- Light Laboratories. All solvents were of HPLC grade. All the reagents were used without further purification. Deionized water, purified by Milli Q system (Millipore, Milford, MA, USA), was used throughout the study. Stock solutions of vitamin C was prepared at 10mg/ml in methanol. All stock solutions were protected from light and stored at low temperature. The stock solutions were further diluted with methanol to give a series of working standards.

Sample preparation:

Within 1 hour of sample collection, the sample was centrifuged at 3000 rpm for 10 minutes and the supernatant collected using Pasteur pipette. The plasma was stored at -70^{o}C until analysis (which was usually within ten days).

Following thawing, vitamin C in plasma was extracted as follows: plasma protein was precipitated with 60% methanol and 1mM EDTA. Plasma (0.4 ml) was mixed with 1.6ml of 60% methanol/EDTA, incubated for 10 minutes at 4^{0}C before centrifuging at 3000 rpm for 20 minutes. The clear phase was transferred to vials until ready for injection.

Instrumentation:

The HPLC analyses was carried out on an Agilent 1100 system composed of a quaternary pump, autosampler, a UV detector and HP chemstation software. The column used for the analyses was an X Bridge TM C18 (150 X 4.6 mm i.d.; 5µm particle size). UV detection was performed at 265nm, while the injection volume was maintained at 20 µL. A degassed isocratic mobile phase containing 0.1% H_3PO_4: ACN (90:10), at a flow rate of 1.0 ml/min and run time of 3 minutes. All chromatographic conditions were carried out at ambient temperature.

Analysis:

The extracted samples were analyzed using the above pre-validated method. Each sample was run and quantified by relating the peak area with those of the serial concentrations of the reference standards. Calibration curves for peak areas versus concentrations of the reference standards were plotted and the obtained data were subjected to regression analysis using the least squares method.

DETERMINATION OF SAMPLE SIZE:

Using values obtained from a previous similar study[11], the sample size was calculated as follows[65]:

$$n = \frac{(u + v)^2 (s_1^2 + s_2^2)}{(m_1 - m_2)^2}$$

where :n= required minimum sample size in *each* group, u= the standard normal deviate (usually 0.84) which corresponds to the power of 80%, v= the standard normal deviate (usually 1.96) which corresponds to the 95% confidence level. S_1= the standard deviation of vitamin C level in the normotensive group (which is 23.27 μmol/L). S_2=the standard deviation of the vitamin C level in the pre-eclamptic group (which is 5.68 μmol/L). m_1= mean vitamin C level in the normotensive group (which is 60.18 μmol/L) m_2= mean vitamin C level in the pre-eclamptic group (which is 45.99 μmol/L)

Substituting, $n = \frac{(0.84 + 1.96)^2 (23.27^2 + 5.68^2)}{(60.18 - 45.99)^2}$ $n = \frac{2.8^2 \times (541.49 + 32.26)}{}$ $n = 22.34$

However, a total sample size of 90 is envisaged, of which 30 will be pre-eclamptic patients and 60 will be normotensive patients.

STATISTICAL ANALYSIS:

The data obtained was analyzed using the Epi Info version 3.3.2 statistical software package. Categorical variables were compared with chi-square test and Fisher exact test as appropriate, while continuous variables were compared using students' t test. A p value of < 0.05 was considered significant.

ETHICS

The study was carried out after obtaining approval from the Health Research Ethics Committee of the Lagos University Teaching Hospital (LUTH). Informed written consent was obtained from the patients prior to sample collection. Laboratory tests were carried out at no cost to the patients.

RESULTS

Ninety (90) women participated in the study. Thirty (30) of these women were pre-eclamptic, while the remaining sixty (60) were normotensive. For each pre-eclamptic patient recruited, two normotensive patients (that were matched for parity) were recruited. All 30 (100%) of the pre-eclamptic patients recruited had features of severe pre-eclampsia.

Of the ninety women recruited, most of the patients were aged between 26 and 30 years (46 patients, accounting for 51.1%), while only 1 patient (1.1%) was aged above 40 years. The mean age was 28.7± 4.4 years (range 21-45 years). (Table 1) Most of the patients were nulliparous (48 patients, representing 53.3% of all patients), while only 3 (3.3%) of the patients recruited had 4 previous deliveries. The mean parity was 1 ± 1.3 (range 0 -4) with the median parity being 0. Thirty nine patients (43.3%) belonged to social class V (i.e. unskilled workers) and 78 (86.7%) were Christian. Eighty eight women (97.8%) were married, while 63 (70%) of all patients recruited were booked patients of LUTH.

TABLE 1: Sociodemographic distributions of all participants

Age	**n (%)**	**(n=90)**	
21-25 years	19 (21.1)		
26-30 years	46 (51.1)	Mean Age	28.7 ± 4.4 years
31-35 years	18 (20.0)		
36-40 years	6 (6.7)		
>40 years	1 (1.1)		
Parity			
0	48 (53.3)		
1	18 (20.0)		
2	6 (6.7)		
3	15 (16.7)		
4	3 (3.3)		
Social class			
I (Professional)	14 (15.6)		
II (Management and Technical)	8 (8.9)		
III (Skilled)	11 (12.2)		
IV (Partly skilled)	18 (20.0)		
V (Unskilled)	39 (43.3)		
Religion			
Christianity	78 (86.7)		
Islam	12 (13.3)		
Marital Status			
Married	88 (97.8)		
Single	2 (2.2)		
Booking status			
Booked	63 (70.0)		
Unbooked	27 (30.0)		

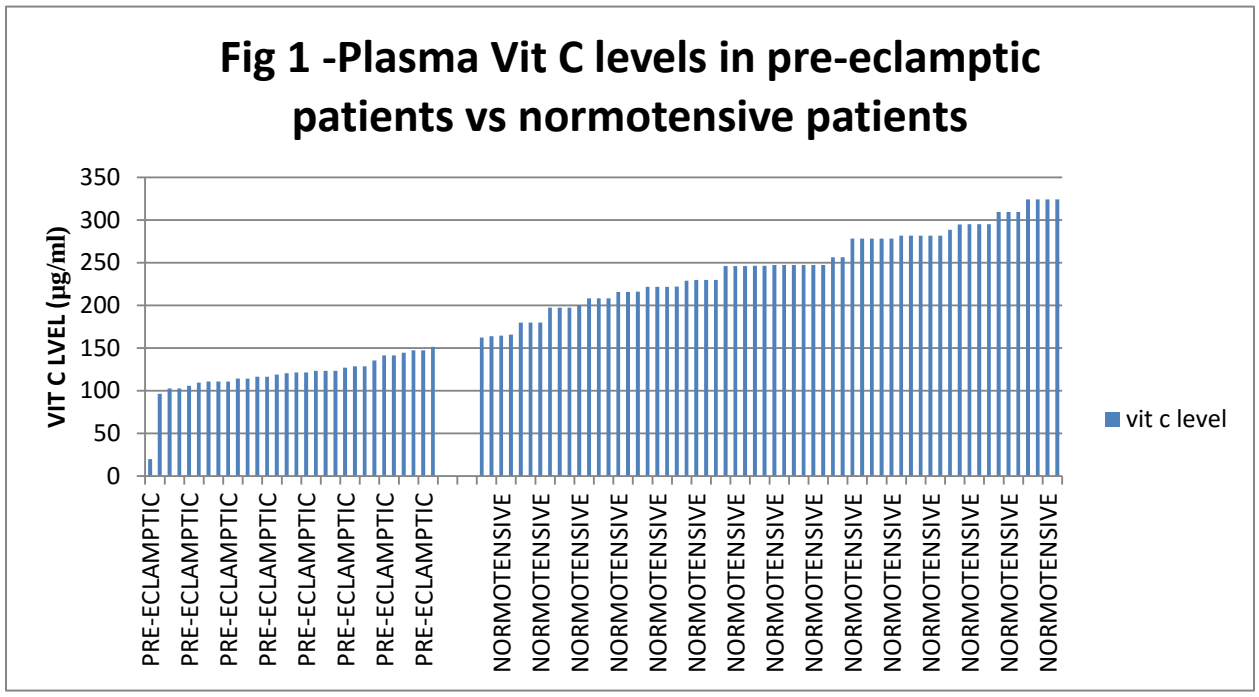
Table 2 shows the socio-demographic features of both groups of patients. The mean age among pre-eclamptic patients was 29.3 ± 4.6 years, whereas the mean age among normotensive patients was 28 ± 4.3 years. There was no statistically significant difference in age between both groups.

Majority of the patients in both groups were nulliparous. Sixteen (53.3%) of the pre-eclamptics and thirty two (53.3%) of the normotensives were nulliparous. Most of the patients in each group belonged to social class V (i.e. unskilled workers): 12 (40.0%) of the pre-eclamptic patients and 27 (45.0%) of the normotensive patients. Twenty five (83.3%) of the pre-eclamptic patients, and 53 (88.3%) of the normotensive group were Christian. Most of the patients were married: 29 (96.7%) of the pre-eclamptics and 59 (98.3%) normotensive patients. Twenty one (70%) of the pre-eclamptic patients were unbooked patients, while only 6 (10%) of the normotensive patients were unbooked. There were no statistically significant associations between pre-eclampsia and parity, social class, religion or marital status. There was, however, a significant association between pre-eclampsia and booking status.

TABLE 2: Sociodemographic characteristics of pre-eclamptic patients vs normotensive patients

Characteristics	Pre-eclamptic (n=30)	Normotensive (n=60)	Test X^2 test	P value
Mean age(years)(SD)	29.3 ± 4.6	28.4 ± 4.3	0.9	0.372
Parity				
0	16 (53.3)	32 (53.3)	0.0	1.000
1	6 (20.0)	12 (20.0)		
2	2 (6.7)	4 (6.7)		
3	5 (16.7)	10 (16.7)		
4	1 (3.3)	2 (3.3)		
Social Class				
I	4 (13.4)	10 (16.7)	1.5	0.830
II	2 (6.6)	6 (10.0)		
III	5 (16.7)	6 (10.0)		
IV	7 (23.3)	11 (18.3)		
V	12 (40.0)	27 (45.0)		
Religion				
Christianity	25 (83.3)	53 (88.3)	0.4	0.520
Islam	5 (16.7)	7 (11.7)		
Marital Status				
Married	29 (96.7)	59 (98.3)	0.3	1.000
Single	1 (3.3)	1 (1.7)		
Booking Status				
Booked	9 (30.0)	54 (90.0)	34.3	<0.001
Unbooked	21 (70.0)	6 (10.0)		

Figure 1 is a clustered bar chart showing the distribution of fasting plasma vitamin C levels in pre-eclamptic and normotensive patients. The mean vitamin C level among the pre-eclamptic patients was 119 ± 23.7 µg/ml (range 19.9 – 151.6 µg/ml). The median vitamin C level for the pre-eclamptic group was 121.1 µg/ml. The mean vitamin C level among normotensive patients was 246.5 ± 45.0 µg/ml (range 162.3 – 324.1 µg/ml), with the median vitamin C level being 246.8 µg/ml.



Mean vitamin C level among pre-eclamptic patients (SD) = 119.3 ± 23.7 µg/ml

Mean vitamin C level among normotensive patients (SD) = 246.5 ± 45.0 µg/ml

T statistic - 14.5. P value - <0.001

Table 3 highlights the pregnancy outcome in both groups. The mean gestational age at delivery in the pre-eclamptic group was 34.6 ± 3.3 weeks (range 29 – 40 weeks). This was significantly lower than the mean gestational age at delivery among normotensive patients (mean =39.0 ± 1.3 weeks, range 32 -40 weeks).

Similarly, the birth weights of babies delivered by pre-eclamptic patients were significantly less than those delivered by normotensive patients. The mean birth weight for the pre-eclamptics was 2.2 ± 0.9 kg (range 1.0 – 4.2 kg); whereas for normotensive patients, the mean birth weight was 3.4 ± 0.5 kg (range 1.8 – 4.55).

Although one (3.3%) pre-eclamptic patient had a stillbirth as compared to none among the normotensive patients, this was not statistically significant. There was a statistically significant association between pre-eclampsia and delivery through caesarean section. Twenty six (86.7%) of the pre-eclamptics were delivered through caesarean section as compared to 20 (33.3%) of the normotensive group.

There was also a significant association between pre-eclampsia and perinatal asphyxia, as evidenced by lower APGAR scores at one and five minutes in babies delivered to pre-eclamptic women when compared to those delivered to normotensive women. Of the 29 pre-eclamptic women that had live births, 10 (27.6%) babies had one minute APGAR scores less than 7, compared to only 5 (8.3%) in the normotensive group. Similarly, 13 (44.8%) of the pre-eclamptic women that had live births had babies with 5 minute APGAR scores less than 7, compared to only 4 (6.7%) in the normotensive group

TABLE THREE: PREGNANCY OUTCOME

Outcome	**Pre-eclamptic (n=30)**	**Normotensive (n=60)**	**Test T test**	**P value**
G.A at delivery range (years)	29 – 40	32 - 40		
Birthweight range(Kg)	1.0 – 4.2	1.8 - 4.55		
Mean gestational age at delivery (SD)	34.6 ± 3.3	39.0 ± 1.3	9.0	<0.001
Mean birth weight (SD)	2.2 ± 0.9	3.4 ± 0.5	8.2	<0.001
Stillbirths				
Yes	1 (3.3)	0 (0.0)	2.0	0.330
No	29 (96.7)	60 (100.0)		
Mode of Delivery				
Caesarean section	26 (86.7)	20 (33.3)	22.8	<0.001
SVD	4 (13.3)	39 (65.0)		
Instrumental	0 (0.0)	1 (1.7)		

Outcome	**Pre-eclamptic (n=29)**	**Normotensive (n=60)**	**Test X^2**	**P value**
APGAR Score(1 minute)				
0 – 2	2 (6.9)	0 (0.0)	14.6	0.002
3 – 4	4 (13.8)	0 (0.0)		
5 – 6	4 (13.8)	5 (8.3)		
7 – 10	19 (65.5)	55 (91.7)		
APGAR Score(5 minutes)				
0 – 2	1 (3.5)	0 (0.0)	19.3	<0.001
3 – 4	2 (6.9)	0 (0.0)		
5 – 6	10 (34.4)	4 (6.7)		
7 – 10	16 (55.2)	56 (93.3)		

DISCUSSION

The present study showed that the plasma concentration of vitamin C was significantly lower in pre-eclamptic patients at LUTH when compared to their normotensive counterparts. This finding is similar to those of researchers elsewhere.[18,23,62] The present study also showed that pre-eclamptic pregnancies were significantly associated with higher frequencies of caesarean deliveries, preterm deliveries, perinatal asphyxia and low birth weight babies than in normotensive pregnancies. These findings regarding the obstetric outcomes in pregnancies complicated by pre-eclampsia are similar to the findings from previous studies.[24-31]

However, the finding from this present study of significantly lower plasma vitamin C levels in pre-eclamptic patients compared to normotensive patients, is at variance with the findings of Bowen RS et al[32] in Durban, South Africa. In their study, placenta, maternal and cord plasma were collected at delivery from twenty nine (29) normotensive pregnant women, twenty one (21) pre-eclamptic and six eclamptic women. Plasma was also collected from 21 non-pregnant matched controls. Levels of ascorbic acid, uric acid, vitamin E, cholesterol, lipid peroxidation products (LPO) and malondialdehyde (MDA) were analyzed by High Performance Liquid Chromatography (HPLC) and colorimetric assay.

In the study of Bowen RS et al, plasma maternal concentration of vitamin C, vitamin E, uric acid, cholesterol and MDA were not significantly different in pre-eclampsia as compared to normal pregnancy. Other findings from their study were that cord plasma concentrations of MDA and vitamin E were significantly higher in eclampsia as compared to pre-eclampsia and normal pregnancy; plasma cord concentrations of LPO and placental concentrations of vitamin E were undetected for normal pregnant, pre-eclamptic and eclamptic women respectively; and uric acid concentrations were significantly increased in eclampsia as compared with normal pregnant controls and pre-eclampsia. The authors concluded that their study did not show any evidence of

deficiency in the maternal protective anti-oxidant systems or of increased production of lipo-peroxidation products in women with pre-eclampsia as compared with normal pregnancy[32].

A possible explanation for this difference in findings regarding plasma vitamin C levels in pre-eclamptics and normotensive pregnant controls could be that certain exclusion criteria in selecting subjects in our study, as well as in some of the other studies[19, 21-23], were not applied in the study by Baker et al. The exclusion criteria in our study included history of use of aspirin (which increases urinary excretion of vitamin C), history of vitamin C supplementation during the antepartum period and history of cigarette smoking (smoking generates free radicals leading to increased utilization of antioxidants including vitamin C, so that smokers have lower levels of plasma vitamin C compared to non-smokers). While it is uncommon in our environment for women to smoke cigarettes, it is more common among women in industrialized nations.

Lower vitamin C levels among pre-eclamptic patients indirectly supports the concept of oxidative stress as a basic pathophysiologic mechanism underlying pre-eclampsia. Vascular endothelial damage is believed to play a key role in pre-eclampsia.[16,33] Free radical mediated lipo-peroxidation may be involved in the endothelial damage seen in pre-eclampsia.[18,23] Excess free radical disturbances are typically accompanied by increased utilization of antioxidants, resulting in a decrease in their concentrations.[23]

Vitamin C forms the first line of anti-oxidant defence in plasma against different types of reactive oxygen species (ROS) and free radicals. As a water soluble vitamin, it works both inside and outside cells to combat free radical damage . In addition, vitamin C contributes to the anti-oxidant activity of lipids by working with gluthathione peroxidase to revitalize vitamin E (a fat soluble anti-oxidant).

The present study also showed that when compared to normotensive pregnancies, pre-eclamptic pregnancies were significantly associated with higher frequencies of caesarean deliveries, prematurity, perinatal asphyxia and low birth weight babies. These findings were in agreement with those of other investigators.[27-31] These outcomes may possibly be explained by the fact that the only definitive cure of pre-eclampsia is delivery of the fetus and the placenta. Because pre-eclampsia is a progressive disorder, delivery is often needed to halt the progression to the benefit of the mother and the fetus.[72] In pregnancies complicated by pre-eclampsia, obstetricians have to balance the desire for achieving in utero fetal maturation with the maternal and fetal risks of continuing with the pregnancy, including progression to eclampsia, abruptio placenta, HELLP syndrome, as well as fetal growth restriction and demise.[32-33]

Delivery is typically recommended for women who develop pre-eclampsia, regardless of disease severity, at or after 37 weeks gestational age.[34] In women with an established diagnosis of pre-eclampsia, delivery should be considered once fetal lung maturity is likely (approximately 32-34 weeks gestation), particularly if either maternal multi-organ involvement or fetal compromise is apparent.[2] Maternal indications for delivery at any gestation include an inability to control hypertension, deteriorating liver or renal function, progressive fall in platelets or neurologic complications.[2] A non-reactive Cardiotocograph (CTG) with decelerations or a fetal condition that is clearly deteriorating often warrants delivery.[2]

As such, pre-eclampsia is the commonest cause of intervention related prematurity accounting for 15% of all premature births and approximately one in five very low birth weight infants (<1500g).[3] Caesarean section is indicated for an already compromised fetus particularly those remote from term, for severe cases of pre-eclampsia with unripe cervixes and where there are obstetric contra-indications to vaginal delivery.[30] The low birth weight (<2.5kg) and low

APGAR scores observed in neonates delivered by pre-eclamptic women may be due to prematurity and fetal growth restriction[35,36]. **LIMITATIONS OF THE STUDY**

1. There is the possibility that some unbooked patients recruited may be chronic hypertensives or have other medical problems prior to the pregnancy without knowing, or may have received inadequate antenatal care, or that patients (booked and unbooked) registered late for antenatal care (such that blood pressure readings and urinalysis prior to 20 weeks gestation are unknown).

   Oxidative stress has been implicated in hypertension(essential), diabetes, cardiovascular disease and chronic inflammatory diseases. Individuals exposed to such oxidative stress have lower vitamin C levels compared to healthy individuals. As such, patients with such medical problems predating the onset of pre-eclampsia (with already lower vitamin C level), may develop a further decline in plasma vitamin C level when pre-eclampsia develops.
2. Patients recruited were not fasted (overnight) prior to blood sample collection. No restrictions on the length of time for abstinence from food, drink, oral medications or intravenous fluids were placed on the patients recruited. By fasting, outside influences on plasma vitamin c concentration may be minimized.

CONCLUSION

The findings from the study suggest that pre-eclampsia is associated with decreased concentration of plasma vitamin C (a potent anti-oxidant), thus supporting the concept of oxidative stress in pre-eclampsia. Unlike the antioxidant enzymes which are synthesized in the body, and whose concentrations cannot be easily influenced[18], antioxidant nutrient levels (such

as vitamin C) can be simply manipulated by dietary or pharmacologic supplementation. The potential role of oxidative stress (and reduced vitamin C levels) in the aetiology of pre-eclampsia should prompt local researchers to investigate the effect of vitamin C supplementation on pre-eclampsia by giving a group of primigravidae vitamin C and another group a placebo, and then subsequently determining the incidence of pre-eclampsia in both groups.

To further demonstrate the presence of oxidative stress in pre-eclampsia, local studies will be required to evaluate other antioxidants (including nutrient antioxidants such as vitamins A and E), with simultaneous evaluation of reactive oxygen species (such as MDA) and lipid peroxide (LPO) levels in pre-eclamptic women and comparing them with the level of these substances in normotensive

REFERENCES:

1. Sibai B, Dekker G. Pre-eclampsia. *The Lancet* 2005; 365(9461): 785-799.
2. Duley L. The management of pre-eclampsia. *The Obstetrician and Gynaecologist* 2000; 2(3): 45-48.
3. Reynolds C, Mabie WC, Sibai BM. Hypertensive states of pregnancy. In: De Cherney AH, Nathan L, editors. Current Obstetric and Gynaecologic Diagnosis and Treatment. 9th Ed. New York: Lange Medical books/McGraw-Hill; 2003. p 338-53.
4. Cudihy D, Lee RV. The pathophysiology of pre-eclampsia: Current clinical concepts. *J Obstet Gynaecol* 2009; 29(7): 576-5.
5. Shennan A. Pre-eclampsia and non-proteinuric pregnancy induced hypertension. In: Luesley DM, Baker PN (eds) Obstetrics and Gynaecology-An evidenced –based text for MRCOG. London, Arnold. 2004; pp 179-186.
6. Shennan A. Hypertensive disorder. In: Edmonds DK, editor. Dewhurst's textbook of Obstetrics and Gynaecology for postgraduates. 7th ed. London: Blackwell Science; 2007. p. 227-235.
7. Redman CWG, Sargent IL. Placental debris, oxidative stress and pre-eclampsia, *Placenta* 2000; 21: 597–602.
8. Sibai BM. Prevention of preeclampsia: a big disappointment, *Am J Obstet Gynecol* 1998; 179: 1275–1278.
9. Uzoigwe SA, John CT. Maternal mortality in the University of Port-Harcourt Teaching Hospital, Port-Harcourt in the last year before the new millennium. *Niger J Med* 2004; 13(1): 32-5.

10. Aboyeji AP, Ijaiya MA, Fawole AA. Maternal mortality in a Nigerian teaching hospital - a continuing tragedy. *Trop Doct* 2007; 37(2): 83-5.
11. Onyiriuka A, Okolo AA. Perinatal outcome in patients with pre-eclampsia in Benin City, Nigeria. *Trop J Obstet Gynaecol* 2004; 21: 148-152.
12. ACOG Committee on Practice Bulletins-Obstetrics ACOG practice bulletin: Diagnosis and management of preeclampsia and eclampsia. *Obstetrics and Gynecology* 2002; 99: 159.
13. Qureshi ZP. Current management of hypertensive diseases in pregnancy. *East Afr Med J* 2002; 79(4): 169-171.
14. Hubeln CA. Oxidative stress in the pathogenesis of preeclampsia, *Proc Soc Exp Biol Med* 1999; 222: 222–235
15. Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia *J Soc Gynecol Investig* 2004; 11: 342–352.
16. Bader TJ, Day LD. Hypertension in Pregnancy. In: Bader TJ, editor. OB/GYN Secrets. 3rd ed. Philadelphia: Elsevier Inc; 2005. 82. p. 212-218.
17. Faas MM, Schuiling GA. Pre-eclampsia and the inflammatory response. *Eur J Obstet Gynecol Reprod Biol* 2001; 95(1): 213-217.
18. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science* 2005; 308: 1592–1594
19. Sagol S, Ozkinay E, Ozener S. Impaired antioxidant activity in women with pre-eclampsia. *Int J Gynecol Obstet* 1999; 64(2): 121-127.
20. Kharb S. Vitamin E and C in pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol* 2000; 93(1): 37-39.

21. Sharma JB, Sharma A, Bahadur A, Vimala N, Satyam A, Mittal S. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. *Int J Gynecol Obstet* 2006; 94(1): 23-27.

22. Paydatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J et al. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *J Am Coll Nutr* 2003; 22(1): 18-35.

23. Llurba E, Gratacos E, Martin-Gallan P, Cabero L, Dominguez C. A comprehensive study of oxidative stress and antioxidant status in pre-eclampsia and normal pregnancy. *Free Radic Biol Med* 2004; 37(4): 557-70.

24. Roes EM, Hendricks JC, Raijmakers MT, Steegers- Theunissen RP, Groenen P, Peters WH et al. A longitudinal study of antioxidant status during uncomplicated and hypertensive pregnancies. *Acta Obstet Gynecol Scand* 2006; 85(2): 148-55.

25. Kaur G, Mishra S, Sehgal A, Prasad R. Alterations in lipid peroxidation and antioxidant status in pregnancy with pre-eclampsia. *Mol Cell Biochem* 2008; 313(1-2): 37-44.

26. Module II: Sampling. In: Varkevisser CM, Pathmanathan I, Brownlee A, editors. Health Systems Research Training Series. Vol 2 Part 1: Proposal Development and Fieldwork. Ontario: International Development Research Centre; 1991. p. 195-220.

27. Panburana P, Phuapradit W, Puchaiwatananon O. Antioxidant nutrients and lipid peroxide levels in Thai pre-eclamptic pregnant women. *J Obstet Gynaecol Res* 2000; 26(5): 377-81.

28. Kishwara S, Tanira S, Omar E, Wazed F, Ara S. Effects of Pre-eclampsia on Perinatal Outcome- A study done in the Specialized Urban Hospital Set Up in Bangladesh. *Bangl Med J* 2011; 40(1): 33-36.
29. Khawaja N, Parveen A, Hussain U, Zahid B, Rehmen R. Frequency and Obstetric Outcome of Hypertensive Disorders of Pregnancy. *Pak J Med* 2001; 10: 22-25
30. Geidam AD, Bako B, Kadas AS, Kullima AA, Kawuma MB. Perinatal outcome in patients with pre-eclampsia at The University of Maiduguri Teaching Hospital, Nigeria. *Kanem J Med Sci* 2008; 2(2): 30-34
31. Ayaz A, Muhammad T, Hussain SA, Habib S. Neonatal outcome in pre-eclamptic patients. *J Ayub Med Coll Abbottabad* 2009; 21(2) 53-5
32. Bowen RS, Moodley J, Dutton MF, Theron AJ. Oxidative stress in pre-eclampsia. *Acta Obstet Gynecol Scand* 2001; 80(8): 719-25
33. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA, Giannone PJ. Maternal pre-eclampsia and neonatal outcomes. *J Preg* 2011; 16:5-12
34. Soydemir F, Kenny L. Hypertension in pregnancy. *Curr Obstet Gynaecol.* 2006; 16(6): 315-320.
35. Oudejans CB, Mulders J, Lachmeijer AM. The parent-of-origin effect of 10q22 in pre-eclamptic females coincides with two regions clustered for genes with down-regulated expression in androgenetic placentas. *Mol Hum Reprod* 2004; 10: 589–598.
36. Buga GA, Lumu SB. Hypertensive disorders of pregnancy at Umtat General Hospital: Perinatal and maternal outcome. *East Afr Med J* 1999; 76: 217-221