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Gestational Antioxidants Reduce Pre-Eclampsia Associated Coagulopathy and Improve Neonatal Outcome

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ABSTRACT

Background: Oxidative stress is blamed in the pathogenesis of pre-eclampsia. However, it is less clear what effect gestational antioxidants would have on pre-eclampsia associated coagulopathy, and on neonatal outcome.

Aim of the Study: To investigate the effect of antioxidants supplementation on coagulopathy during pre-eclampsia and to evaluate maternal and neonatal outcome.

Patients and Methods: The study was performed as a randomized, controlled, blinded trial; 251 high risk pregnant women were randomized to receive either antioxidants (1000mg vitamin C, 400IU vitamin E, 100µg Selenium and 1500IU vitamin A) or placebo. Primary maternal outcome was pre-eclampsia or one of its complications. Newborns for both groups were followed through the neonatal period. In each trimester, detailed blood chemistry lipogram, and coagulation profile were done. Antioxidants blood levels (vitamins A, C, and E) were measured immediately before delivery.

Results: Incidence of pre-eclampsia did not differ between the two groups. However, a significant reduction in disease severity was noticed. Antioxidants reduced the levels of D-dimer, von Willibrand factor, and fibrinogen significantly. Platelets activity showed a significant reduction in the supplemented group. Antioxidants were significantly higher in blood of the supplemented group. Concerning neonatal outcome, low birth weight, need for neonatal intensive care and neonatal hyperbilirubinaemia were significantly reduced in newborns of the supplemented group.

Conclusion: Supplementing high risk women with antioxidants during pregnancy may help to counteract the oxidative stress and control coagulopathy. However, it does not prevent the disease. This study suggests potential benefits for gestational antioxidants as regards neonatal outcome in pre-eclampsia.

Key Words: *Coagulopathy – Antioxidants – Pre-eclampsia – Neonatal jaundice – Vitamins.*

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INTRODUCTION

Pre-eclampsia is a complex multisystem disorder that affects 2-8% of all pregnancies and causes about 30% of all maternal deaths **[1]**. Despite all effort, the only successful therapy, once the diagnosis of pre-eclampsia has been established, is termination of pregnancy. Accordingly, pre-eclampsia remains a major cause of several neonatal problems, such as, preterm birth, intrauterine growth restriction (IUGR), intrauterine hypoxia and perinatal death **[2]**.

The cause of pre-eclampsia is not fully understood; however, several environmental, nutritional and genetic factors have been suggested to trigger pre-eclampsia through initiating placental ischemia and endothelial cells dysfunction. Pathologically, pre-eclampsia is characterized by vasoconstriction, cell damage and coagulation disorders **[3]**.

During normal pregnancy, hemostasis shifts in the direction of hypercoagulability, thus decreasing bleeding complications during delivery, and in this aiding the uterine muscle contraction, the primary factor responsible for interrupting blood flow **[4,5]**. In this regard several noticeable changes in the hemostatic balance have been noticed. Thrombocytopenia, increased endogenous thrombin generation, acquired activated protein C resistance, decreased activated partial thromboplastin time (aPTT) and increased prothrombin complex level with international normalized ratio of less than 0.9 have been reported **[6]**. With the exception of factor XI, most coagulation factors including fibrinogen are increased

during normal pregnancy **[4,7]**. Although platelet count remains within the normal range during the first and second trimesters, benign gestational thrombocytopenia (80-150 X 10^9/L) can be observed in the third one. This is associated with activation of platelets, release of beta-thromboglobulin and platelet factor-4, and an unchanged bleeding time **[8]**. The level of both plasminogen activator inhibitor-1 from endothelial cells and plasminogen activator inhibitor-2 from placenta are increased. Prothrombin fragment 1+2, TAT complex, soluble fibrin and D-dimer increase as well. All these reflect activation of blood coagulation and simultaneous increase in fibrinolysis, which normalize 4-6 weeks after delivery without signs of organ dysfunction **[7,8]**.

Any factor that causes a change in the delicate hemostasis balance during pregnancy can exaggerate hypercoagulability state and increase risk of disseminated intravascular coagulopathy (DIC) and multiorgan dysfunction. This is observed in situations like pre-eclampsia, eclampsia, and intrauterine fetal death **[9,10]**. It has been noticed that an increase in oxygen free radicals is associated with exaggeration of hypercoagulability state **[11]**, suggesting a compromise in antioxidant capacity in such situations. Indeed, lipid peroxidation resulting from endothelial damage during pre-eclampsia leads to an increased very low density lipoprotein and low antioxidants level **[12]**.

On the other hand, several neonatal problems that largely influence neonatal mortality and morbidity, such as chronic lung disease, necrotizing enterocolitis, retinopathy of prematurity and intracranial hemorrhage, are thought to be related to the action of reactive oxygen species, especially in preterm infants **[13]**. It is thought that neonates, especially prematurely born, have an overstressed underdeveloped antioxidant system. Neonates depend on maternally transferred antioxidants that do not cross the placenta in sufficient amount until the third trimester of gestation **[14]**. In pre-eclampsia maternal antioxidant system is exhausted and there is a higher risk of preterm delivery. Another neonatal condition that is thought to be related to oxidative stress in neonates is neonatal idiopathic hyperbilirubinaemia **[15]**. It was proven that the decrease in plasma bilirubin was contemporary with an increase in plasma antioxidant capacity and decrease in oxidative stress in preterm infants **[16]**.

Despite the fact that several hematological and biochemical studies suggested that oxidative stress may be involved in the pathogenesis of pre-eclampsia and its effects **[17-19]**, it is less clear whether intake of antioxidant vitamins can protect against pre-eclampsia and improve neonatal outcome. On one hand, an observational study had shown an increased risk of pre-eclampsia among women with an intake of vitamin C below the recommended dietary allowance **[20]**, and another randomized controlled trial had shown a lower occurrence of pre-eclampsia in high-risk women who were supplemented with high doses of the antioxidative vitamins C and E **[21]**. On the other hand, other studies failed to show any difference in the incidence of pre-eclampsia between treated and untreated women **[22,23]**. The effect regarding neonatal outcome was most confusing as one trial showed an increase in the rates of low birth weight in the antioxidant supplemented group **[22]**.

Our aim was to study the potential effects of antioxidants supplementation on coagulopathy-associated pre-eclampsia and its effect on both maternal and neonatal outcomes.

PATIENTS AND METHODS

Study population:

Study population was recruited form Sohag University Hospital obstetric outpatient clinic in the period from July 2006 to June 2008. Only pregnant women with high risk for pre-eclampsia were considered. The inclusion criteria were gestational age between 6-10 weeks with one or more of the following risk factors: pre-eclampsia in the pregnancy preceding the current one, eclampsia in any previous pregnancy, essential hypertension requiring medication, diagnosis of HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), and chronic renal disease pre-pregnancy or during pregnancy. Exclusion criteria included maternal liver disease, diabetes mellitus and possible materno-fetal fetus Rh incompatibility.

The assigned ladies were counseled about their participation in the study. Written informed consent was obtained prior to recruitment. Women had the right to refuse to participate and/or

withdraw from the study at any time without being denied or their babies for regular full clinical care. Personal information and medical data collected were confidential and were not made available to a third party. After delivery, newborns were followed-up and included in the study.

Study design and protocol:

This study was a prospective randomized controlled blinded trial. Participating women were randomly assigned to receive either antioxidants cocktail (group I) containing 1000mg vitamin C, 400IU vitamin E, 100µg Selenium and 1500IU vitamin A; or identical placebos (group II). This supplementation was given daily from enrolment to delivery and was continued even after pre-eclampsia or hypertension was diagnosed. Participating women were seen once a month for clinical evaluation according to the standardized antenatal care protocol. The definition of pre-eclampsia was in accordance with the American College of Obstetricians **[2]**. In the last visit in the first and second trimester, and immediately before delivery blood samples were drawn for laboratory investigations.

A total of 251 women consented to the study of which 126 women were recruited to the antioxidant arm (group I) and 125 to the placebo (group II). Of group I, 105 women were followed up until delivery while 21 women were lost during follow-up (7 abortions and 14 did not come for antenatal care). Of group II, 102 women were followed-up until delivery while 23 women were lost to follow-up (10 abortions and 13 did not come for antenatal care). Blood sample for hematological and biochemical testing were drawn from 126 women in group I in the first trimester (group Ia), 110 women in the second trimester (group Ib), and 105 women in the third trimester (group Ic). For group II, blood samples were drawn from 125 women in the first trimester (group IIa), 114 women in the second trimester (group IIb), and 102 women in the third trimester (group IIc). Vitamins levels were done only in the last sample drawn in the third trimester.

Routine investigations included complete blood count (CBC) done on H-Max Coulter system, coagulation profile including prothrombin time (PT) and activated partial thromboplastin time (aPTT) on Sysmex Dade Behring fully automated system; random blood glucose, liver function tests, renal function tests, lipogram [cholesterol, triglycerides (TG), high density lipoprotein (HDL-c) and low density lipoprotein (LDL-c)] on Beckman-Synchrone CX-9 fully automated chemical autoanalyzer; and urine for protein. Research investigations including plasma fibrinogen, thrombin time (TT), and D-dimer done on Sysmex Dade Behring fully automated system; von Willibrand Factor (vWF) by latex agglutination method supplied by Dade Behring; platelet activation by ADP/Collagen on PFA-100 Dade Behring; and vitamins assay including vitamins A and C by high performance liquid chromatography (HPLC) and vitamin E assay by gas chromatography **[24]**.

All newborns were evaluated by detailed full clinical examination. Neonates were followed-up every week for the first four weeks of life or until discharge from neonatal care if the period of admission to neonatal care extended beyond the first four weeks of life. Neonatal outcomes were recorded. Neonates were grouped into two groups, group I, whose mothers received antioxidants during pregnancy; and group II, whose mothers did not receive antioxidants during pregnancy. Of the newborns included in the study, 98 were followed-up in group I for the whole neonatal period while only 90 newborns completed the neonatal follow-up in group II. Blood sample was drawn whenever clinical jaundice was apparent. A total bilirubin level above 13mg/dl was used to diagnose neonatal hyperbilirubinaemia.

Details of blood sampling and laboratory technique:

The pregnant females were sampled before breakfast on light dinner. Blood was withdrawn in appropriate vacutainers for different tests. For the coagulation profile, the citrated samples were centrifuged in cooling centrifuge system at 4000 rpm for 10min at 20°C to prepare platelet poor plasma, then the plasma was delivered to SYSMEX DADE BEHRING Fully Automated system for PT, aPTT, TT, fibrinogen by clotting method and D-dimer by turbidimetric method, Kits were supplied by DADE BEHRING. Normal values were as follows: PT 10.5-13sec., aPTT 26-38sec., TT 14-17sec., fibrinogen 180-360mg/L, D-dimer <20µg/dL. vWF was assayed by latex agglutination method supplied by DADE BEHRING with a normal value of 70-150%. For platelets activation, the second citrated tube was delivered to PFA-100 DADE

BEHRING as whole blood platelets activation, using cartilage for ADP/Collagen, normal closure time is up to 175sec. For blood picture the K-EDTA blood tube was delivered to H-Max Coulter system fully automated system.

For vitamins assays the heparinized tube was rapidly centrifuged at 4°C and immediately separated in the dark and kept at –70°C for HPLC. Vitamins assays were done according to previously published method **[24]**. Vitamin A assay was carried out using a stainless steel column 0.125m long and 4mm in internal diameter packed with octa-decylsinlyl silica gel for chromatography R (5µm). Flow rate used was 1mL/min, with detector of spectrophotometer set at 325nm and retention time of 3min. The reference range was 0.35-0.75µg/dL. Vitamin C assay was carried out using 0.8mL/min flow rate at an ambient temperature with a detection of 254nm and retention time of 6min. The reference range was 280-1100mmol/L. Vitamin E assay was carried out using a fused silica column 30mm long and 0.25mm in internal diameter using helium as a carrier gas. Flow rate used was 1mL/min at split ratio of 1:100. Temperature used was 280°C for the column and 290°C for the injection port and detector. Run time was adjusted at twice the retention time (15min). The reference range was 5-20µg/mL.

Main outcome measures:

Our maternal primary outcome was the occurrence of one or more of the following: Pre-eclampsia, severe pre-eclampsia (defined as severe gestational hypertension plus proteinurea), delivery for pre-eclampsia at or before 34 weeks' gestation, eclampsia, HELLP syndrome and severe proteinuria defined as excretion of 5000mg or more of protein over 24h. The neonatal outcomes were preterm delivery before 37 weeks' gestation, low birth weight (<2500g), small for gestational age (<10^{th} centile of the WHO recommended standard **[25]**), need for neonatal intensive care admission and neonatal death before hospital discharge.

Statistical analysis:

Statistical analysis was performed using SPSS software, version 10 (SPSS, Chicago). Only ladies and newborns, who completed the follow-up were included in the analysis of outcome. Summary data were presented by group as number (%) or mean (SD) and range when appropriate. We presented outcomes analyses (maternal and neonatal) as simple risk ratios with 95% CIs. The independent sample *t*-test was used to assess the significance of the difference between continuous variables in the two groups. The χ^2 test or the Fisher exact test was used to assess the statistical significance of categorical variables. $p<0.05$ was considered statistically significant.

RESULTS

Baseline characteristics and distribution of risk factors showed no significant differences at study entry between ladies recruited in the two groups (Table 1). The most common risk factors were similar in both groups, being chronic hypertension (39.9% and 34.4) followed by a history of pre-eclampsia (31.8% and 33.6). Multiple risk factors were seen in 20.6% and 18.4% of cases followed-up until delivery in group I and group II, respectively.

Table (2) showed the maternal outcomes for the pregnant women who were followed-up until termination. Pre-eclampsia was seen in 19 (18.09%) versus 22 (20.95%) women. Treatment with antioxidant did not reduce this risk. The risk of severe pre-eclampsia and HELLP syndrome was significantly lower in the supplemented group ($p=0.02$ and 0.04, respectively). Early onset pre-eclampsia (delivery for pre-eclampsia <34 weeks' gestation) was similar between both groups. Three (2.85%) women taking antioxidant developed severe proteinurea compared with five (4.90%) on placebo, without any significant difference among both groups. Other serious morbidities were similar in antioxidant versus placebo groups including eclampsia and maternal admission to intensive care unit.

Among the neonatal outcome (Table 3) all preterm deliveries, small for gestational age tend to be similar in both groups, while maternal supplementation with antioxidant significantly reduced the occurrence of low-birth weight, admission to NICU and neonatal hyperbilirubinaemia ($p=0.02$, 0.004 and 0.003; respectively). Gestational age, birth weight and age at time of blood sampling of newborn infants, who developed hyperbilirubinaemia, were similar in both groups. A significant ($p<0.05$) decrease in the serum level of creatinine, cholesterol and LDL-C in the supplemented group was observed only in the third trimester (Table 4).

Hematological and coagulation variables in the study groups were shown in Table (5) and Fig. (1). The hematocrite showed a significant (p<0.05) decrease in the supplemented group in 3rd trimester when compared to the placebo group of the same trimester. Platelet count showed a highly significant (p<0.005) decrease in the 3rd trimester of both supplemented and non supplemented placebo groups when compared to their corresponding groups of 1st trimester. Platelet activity reflected by closure time on PFA-100 showed a highly significant (p<0.005) reduction (as shown by the significant increase in time) in the supplemented group in 2nd and 3rd trimesters when compared to the control (placebo) group for the same trimester. Within the non-supplemented group there was highly significant (p<0.005) increase in platelet activity reflected by the decrease in closure time when the 1st trimester group was compared to both 2nd and 3rd trimester groups. Fibrinogen, vWF and D-dimer levels showed a steady increase in both supplemented and placebo groups as we proceed through pregnancy from 1st, 2nd to the 3rd trimester. However, the supplemented group showed lower levels of the three parameters when compared to placebo group in the same trimester. This reached significance for Fibrinogen and D-dimer (p<0.005 for 1st and 3rd trimesters and p<0.05 for 2nd trimester).

Table (6) showed the antioxidant levels in the third trimester in both groups. There is a significant increase in the level of all of vitamins supplemented (p<0.05 for vitamin A, p<0.003 for vitamin C and p<0.05 for vitamin E). The correlations between antioxidants levels with hematological variables and lipogram in supplemented group are given in Table (7). Significant correlations were found between vitamin A level and both leukocytic count (p=0.002) and D-dimer (p=0.003). Also vitamin C correlated significantly with fibrinogen level (p= 0.004), D-dimer (p=0.002), ADP/collagen closure time (p=0.002) and LDL-C (p=0.004), while vitamin E level correlated significantly (p=0.005) to both fibrinogen and D-dimer and to ADP/collagen closure time (p=0.003).

Table (1): Base line characteristic and distribution of risk factors in the studied groups.

	Group I (n = 126)	Group II (n = 125)
Age (years)	29.3±6.2	30.6±7.4
Parity:		
Primipara	88 (69.9%)	81 (64.8%)
Multipara	38 (30.1%)	44 (35.2%)
Body mass index (BMI)	30.6±5.8	30.2±4.5
Chronic hypertension	49 (39.9%)	43 (34.4%)
Previous pre-eclampsia	40 (31.8%)	42 (33.6%)
Previous eclampsia	5 (4.0%)	7 (5.6%)
Previous HELLP	10 (7.9%)	8 (6.4%)
Previous chronic renal disease	3 (2.4%)	2 (1.6%)
Multiple pregnancy	11 (8.7%)	8 (6.4%)
BMI >30	24 (19.0%)	21 (16.8%)
Multiple risk factors	26 (20.6%)	23 (18.4%)

Data are presented as mean ± SD or number (%) as appropriate, No significance differences between any variable could be detected.

Table (2): Maternal outcome for the cohort who completed the follow-up.

	Group I (n = 105)	Group II (n = 102)	Risk ratio (95% CI)	p-value
Pre-eclampsia	19 (18.09%)	22 (20.95%)	0.839 (0.498-1.454)	0.53
Severe pre-eclampsia	3 (5.71%)	12 (7.84%)	0.242 (0.070-0.835)	0.02
Gestational hypertension	8 (7.61%)	10 (9.80%)	0.777 (0.319-1.890)	0.57
Eclampsia	1 (0.95%)	3 (2.94%)	0.328 (0.034-3.062)	0.32
HELLP	1 (0.95%)	8 (7.84%)	0.121 (0.015-0.953)	0.044
Delivery related to pre-eclampsia (<34 wks)	5 (4.67%)	6 (5.88%)	0.809 (0.255-2.569)	0.72
Severe proteinurea	3 (2.85%)	5 (4.90%)	0.582 (0.143-2.375)	0.45
Maternal admission to intensive care unit	2 (1.9%)	5 (4.90%)	0.400 (0.079-2.014)	0.27
Maternal mortality	0	1 (0.98%)	0.333 (0.013-7.860)	0.50

Data are presented as number (%).

Table (3): Neonatal outcomes of therapy the cohort who completed neonatal follow-up.

	Group I (n = 98)	Group II (n = 90)	Risk ratio (95% CI)	p-value
Preterm delivery (<37wks)	3 (3.06%)	8 (8.88%)	0.344 (0.094-1.258)	0.16
Low birth weight <2500gm	17 (18.88%)	29 (32.22%)	0.538 (0.318-0.910)	0.02
Admission to neonatal intensive care unit	4 (4.08%)	16 (17.77%)	0.229 (0.079 0.661)	0.004
Hyperbilirubinaemia	11 (11.2%)	32 (35.6%)	0.315 (0.169-0.588)	0.003

Data are presented as number (%).

Table (4): Blood chemistry in the studied groups.

	First trimester		Second trimester		Third trimester	
	Group Ia (n = 126)	Group IIa (n = 125)	Group Ib (n = 110)	Group IIb (n = 114)	Group Ic (n = 105)	Group IIc (n = 102)
Serum albumin (g/dL)	4.2±0.98 (3.8-5.5)	4.0±1.1 (3.2-5.3)	3.9±1.23 (3.3-5.4)	3.9±1.1 (3.2-5.1)	3.1±0.77 (3.2-4.9)	3.1±0.87 (2.8-4.6)
Serum creatinine (µm/L)	87.39±39 (73-120)	101.8±22.4 (74-150)	99.5±25.93 (61-156)	109.26±29.61 (55-157)	88.9±19.14* (58-131)	125.15±23.55* (69-163)
Uric acid (mg/dL)	3.4±0.59 (3-5.2)	3.92±0.72 (2.1-5)	4.13±0.58 (3-5.1)	4.4±0.63 (2.7-5.1)	4.3±0.69 (2.3-5.3)	4.6±0.66 (2.8-5.5)
Cholesterol (mg/dL)	175.48±35.9 (131-242)	184.1±23.4 (126-231)	185.8±35.76 (146-260)	193.83±27.88 (140-260)	196.3±24.61* (150-247)	226.45±37.08* (160-310)
Triglycerides (mg/dL)	142.69±40.6 (79-228)	139±38.85 (60-218)	142.8±40.6 (79-228)	140.73±47.92 (72-280)	146.9±42.36 (70-243)	152.87±40.62 (80-228)
LDL-C (mg/dL)	102.69±40.6 (54-128)	89.22±9.09 (70-110)	87.6±15.39 (65-128)	97.76±13.28 (78-123)	91.2±16.12* (65-130)	122.27±91.81* (79-165)
ALT (IU/L)	26.76±8.43 (23-44)	27.55±9.87 (20-46)	34.56±6.87 (22-44)	35.54±7.56 (25-46)	45.47±21.48 (31-100)	48.5±28.63 (33-160)
AST (IU/L)	22.76±5.44 (18-33)	30.43±5.87 (25-45)	32.4±7.43 (18-45)	35.87±7.55 (26-48)	42.66±18.65 (28-80)	44.32±24.52 (30-140)
Serum Bilirubin (mg/dL)	0.44±0.035 (0.4-0.8)	0.43±0.098 (0.5-0.55)	0.52±0.090 (0.5-0.63)	0.62±0.07 (0.6-08)	0.73±0.078 (0.7-2.8)	0.77±0.085 (0.6-3.2)

Data are presented as mean ± SD (range), *p<0.05.

Table (5): Hematological and coagulation variables in the studied groups.

	First trimester		Second trimester		Third trimester	
	Group Ia (n = 126)	Group IIa (n = 125)	Group Ib (n = 110)	Group IIb (n = 114)	Group Ic (n = 105)	Group IIc (n = 102)
Leukocytes ($X10^9$/L)	7.6±2.16 (4.6-13)	7.89±1.49 (4.7-11)	7.65±2.16 (4.6-13)	7.48±1.68 (4.6-119)	8.61±2.11 (5.4-149)	8.58±2.1 (5.4-139)
Hemoglobin (g/dL)	12.02±1.24 (9-15)	11.88±0.91 (9-14)	11.43±1.15 (9-14.3)	12.18±1.0 (10.4-14.2)	11.9±1.55 (9-15)	14.79±1.33 (9-15)
Hematocrite (%)	36.43±4.8 (26-47)	35.36±3.83 (30-43)	36.45±4.55 (27-47)	37.4±4.41 (27-47)	37.46±4.07* (32-47)	47.27±17.08* (32-54)
Reticulocytes (%)	1.07±0.4 (0.5-2.8)	1.03±0.34 (0.5-2)	1.44±0.59 (0.5-3)	1.34±0.62 (0.5-3)	1.21±0.56 (0.5-3)	1.28±0.68 (0.5-3.3)
Platelets ($X\ 10^9$/l)	270±84.9** (143-432)	267.4±74.26** (155-432)	263±83.4 (140-432)	235.7±47.5 (155-334)	219.7±65.8** (100-378)	220.5±65.8** (90-376)
ADP/Collagen Closure Time (sec.)	101.4±4.5 (82-159)	98.7±35.9** (69-179)	104.4±28.7** (94-170)	75.5±12.8** (65-130)	108.4±27.4** (90-175)	75.5±19.6** (58-126)
PT (sec.)	12.5±1.27 (10.4-15)	11.73±0.81 (10.4-13)	12.26±0.12 (10.4-14.6)	11.75±0.68 (10-12.8)	12.12±.15 (10.3-19)	11.99±3.14 (10.3-22)
aPTT (sec.)	35.2±3.8 (27-41)	33.92±3.81 (27-41)	34.22±3.95 (27-41)	33±3.33 (27-38)	34.37±6.68 (27-50)	34.37±7.73 (27-55)
TT (sec.)	14.89±1.53 (13-19)	13.86±0.93 (13-16)	14.32±1.43 (12-17)	13.99±0.82 (12-15.7)	14.84±1.82 (11-19)	14.7±1.71 (11-19)
Fibrinogen (mg/dL)	337.56±62.99** (200-450)	361.86±32.38** (270-420)	375.4±49.49* (250-450)	430.3±40.9** (330-540)	390.6±75.23** (180-460)	480.25±88.7** (150-520)
vWF (%)	87.7±10.37 (70-120)	90.83±10.32* (80-120)	94.47±15.21 (70-130)	101.03±13.74* (75-125)	90.24±14.52 (70-130)	105.7±14.79* (70-130)
D-dimer (µg/dL)	28.33±12.19** (10-59)	34.15±18.88** (18-70)	35.17±12.96* (10-70)	46.43±16.87* (26-109)	44.48 ±4.52** (10-76)	55.88±0.66** (40-100)

Data are presented as mean ± SD (range), *p<0.05, **p<0.005, (for platelets count Ia versus Ic and, IIa versus IIc; for ADP/collagen closure time Ib versus IIb, Ic versus IIc, and IIa versus IIb and IIc; for Fibrinogen Ia versus Ib, Ia versus Ic, IIa versus IIb, IIa versus IIc; for vWF IIa versus IIb, and IIa versus IIc; for D-dimer Ia versus Ib, Ia versus Ic, IIa versus IIb, and IIa versus IIc).

Table (6): Antioxidants level in the 3rd trimester.

	Group Ic n(105)	Group IIc n(102)	*p*-values
Vitamin A (µg/dL)	0.43±0.06 (0.26-0.90)	0.33±0.09 (0.17-0.63)	<0.05
Vitamin C (mmol/L)	650.38±144.19 (430.65-1080)	209.9±76.64 (237.7-548.5)	<0.003
Vitamin E (µg/mL)	12.5±5.3 (6.3-20.2)	8.5±3.6 (6.8-13.4)	<0.05

Data are presented as mean ± SD (range).

Table (7): Correlation between the antioxidant levels (vitamin A, C and E) and various hematological and lipogram values in antioxidants supplemented group ladies.

	Hemoglobin (g/d)	Platelet (n)	Leukocytes (n)	Fibrinogen mg/dL	D-dimer µg/dl	vWF (%)	ADP/collagen Closure time (s)	Cholesterol (mg/dL)	TG (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)
Vitamin A (µg/dl)	*r*=0.090 *p*=0.19	*r*=0.326 *p*=0.08	*r*=0.655 *p*=0.002	*r*=0.138 *p*=0.10	*r*=0.745 *p*=0.003	*r*=0.089 *p*=0.23	*r*=0.089 *p*=0.12	*r*=0.328 *p*=0.09	*r*=0.090 *p*=0.07	*r*=0.260 *p*=0.08	*r*=0.140 *p*=0.09
Vitamin C (mmol/L)	*r*=0.212 *p*=0.06	*r*=0.143 *p*=0.09	*r*=0.232 *p*=0.06	*r*=0.750 *p*=0.004	*r*=0.649 *p*=0.002	*r*=0.213 *p*=0.08	*r*=0.723 *p*=0.002	*r*=0.098 *p*=0.08	*r*=0.087 *p*=0.10	*r*=0.750 *p*=0.004	*r*=0.250 *p*=0.35
Vitamin E (µg/mL)	*r*=0.354 *p*=0.06	*r*=0.275 *p*=0.08	*r*=0.135 *p*=0.27	*r*=0.627 *p*=0.005	*r*=0.680 *p*=0.005	*r*=0.245 *p*=0.230	*r*=0.834 *p*=0.003	*r*=0.095 *p*=0.21	*r*=0.210 *p*=0.09	*r*=0.200 *p*=0.56	*r*=0.095 *p*=0.06

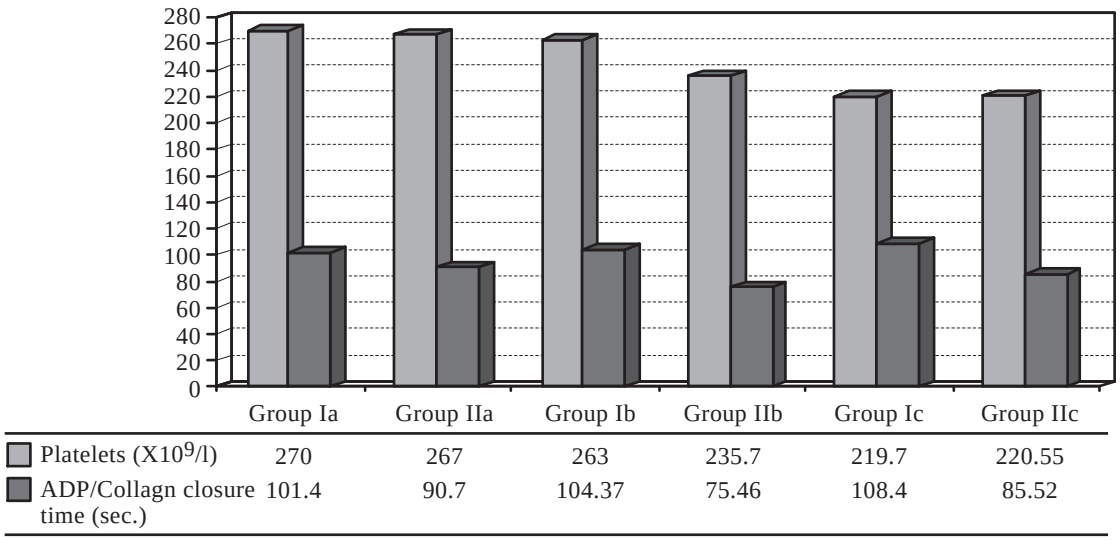

Fig. (1): Platelet count and platelet activation in the studied groups.

DISCUSSION

Thrombotic and bleeding complications of pre-eclampsia are the leading causes of maternal morbidity and mortality with hypofibrinogenaemia and DIC being the most serious complications **[26]**. Early termination of pregnancy leads to fetal loss; however, when the pregnancy is allowed to continue with the usual treatment of pre-eclampsia, intrauterine hypoxia may result into fetomaternal complications as gestational hypertension, IUGR or fetal deaths **[16]**. Thus, though termination of pregnancy would limit maternal complications, the neonatal outcome whether pregnancy was terminated or allowed to continue is in fact limited in this

disease. Therefore, searching for a way to improve placental function in pre-eclamptic mothers seems to be the only way to improve neonatal outcome in such condition. It was shown that when the placental function is improved with healthy functioning endothelium the fetal blood flow increases with possible less complications for mother and fetus **[27]**.

Possible contributing factors for development of pre-eclampsia may be the presence of excessive amounts of free radicals, and a decline in natural body antioxidant enzymes **[9]**, that lead to endothelium dysfunction, with subsequent platelets activation and adhesion and finally the beginning of DIC **[14]**. Antioxidants, such as vitamin C, vitamin E, selenium and carotenoids, can neutralize free radicals **[28]**. The current randomized controlled study evaluated the effect of anti-oxidants (vitamin A, C, E and selenium) in pregnant women at risk for pre-eclampsia. We hypothesized that maternal supplementation with these combined antioxidants in a group of population at risk for pre-eclampsia might decrease the risk for the development of pre-eclampsia and its complications and improve the neonatal outcomes.

Indeed in this study supplementation of antioxidants, although reduced the severity of pre-eclampsia, it did not prevent it; however, the most pronounced effect was on the neonatal outcome. The neonatal outcome showed marked improvement in the supplemented group. This was reflected as a reduction in number of low birth weight and admissions to neonatal intensive care unit with marked improvement in the incidence of neonatal hyperbilirubinaemia. This agrees with the previous studies that showed an increased neonatal tolerance to stress and improvement of neonatal outcomes if pretreatment with antioxidants such vitamins C, E and carotenoids was given in a model of neonatal sepsis **[29]**. In animal studies this was shown to be caused by antioxidants ability to correct the endothelium insufficiency with recovery of the fetal circulation **[27]**. Also, our finding that the incidence of neonatal jaundice was decreased in neonates whose mothers were supplemented with antioxidants during pregnancy suggests that neonatal antioxidant capacity improved through maternal supplementation of antioxidants. This agrees with the previous studies in which antioxidants were shown to correct destruction of erythrocytes-deficient glucose-6-phosphate dehydrogenase enzyme, thus, reducing hemolysis and hyperbilirubinaemia **[10]**.

Our data could not confirm that maternal supplementation with anti-oxidant can reduce the risk of pre-eclampsia when compared to the control group and this result agrees with that of both Rumbold et al. **[30]** and Spinnato et al. **[23]**. However, this finding contradicts the findings of Chappell and his colleagues **[21]**, who reported a reduction in pre-eclampsia in high risk women who were supplemented with antioxidants. Nevertheless, the risk of severe pre-eclampsia and HELLP syndrome was significantly decreased in the current study. In this regard, our findings are compatible with that of Rumiris et al. **[31]**.

In the present study the supplemented group showed a significant reduction in values of D-dimer, which means a reduction in the degree of coagulopathy. This finding is in agreement with the work of Rumiris et al. **[31]**. vWF which has a large multimeric structure that increases the adhesive property to the platelets and endothelium and thus acting as a nidus for thrombosis, showed a tendency to decrease in antioxidants supplemented group. This combined with the highly significant reduction in platelets activity in the supplemented group reflects a marked reduction in platelet-endothelial interaction, which is responsible for coagulopathy. Also vWF reduction is a very important indicator for improvement of the inflammatory process associated with the placental endothelium dysfunction, as it is one of the acute phase reactants. These findings are in agreement with the findings of both Rumbold et al. and Sibai et al. **[11,23]**. Both suggested that antioxidants may improve placental endothelium insufficiency and cause improvement of fetomaternal circulation.

Our study showed a significantly lower fibrinogen level in the supplemented group. This further supports our suggestion that antioxidants reduced hypercoagulability state in those patients. Fibrinogen is considered the most important member of the acute phase reactants family and its reduction together with the reduction in the D-dimer level indicates that inflammation is somewhat controlled and thus possibly controlling pathogenesis of DIC. These findings were previously reported **[4,9]**. In the placebo non supplemented group, the plasma

fibrinogen showed an increase to the hypercoagulation level; this together with presence of high vWF level and activated platelets promote thrombosis. However, it was also noticed in our study that hypofibrinogenaemia occurred in complicated cases regardless of being supplemented or not. This could be explained by the occurrence of DIC, which agrees with other authors **[1,8]**. Our study reported a significant reduction in the platelet count in both supplemented and placebo. This reduction was marked in the complicated cases denoting possible increase in pathogensis of DIC.

The plasma levels of cholesterol and low density lipoprotein were significantly lower in the supplemented group with a significant negative correlation between LDL-C and vitamin C level (p=0.004). This may suggest that placental production of lipid peroxides is abnormally increased in pre-eclampsia. The reason for this is not clear, but if placental antioxidant enzymes were deficient, lipid peroxides would increase unchecked. This explanation was suggested by Chappell et al. **[12]**.

In conclusion supplementing high risk ladies with antioxidants during pregnancy may help to counteract the oxidative stress and control hypercoagulability state that are blamed in the pathogenesis of pre-eclampsia. However, the only maternal clinical benefit of this seems to be reducing the severity of pre-eclampsia rather than preventing the disease. This study suggests potential benefit for gestational antioxidants as regards neonatal outcome in pre-eclampsia that may exceed maternal benefit.

REFERENCES

1- Redman CW, Sargent IL. Latest advances in understanding pre-eclampsia. Science. 2005; 308: 1592-94.

2- ACOG practice bulletin. Diagnosis and management of preeclampsia and eclapmsia. Int J Gynaecol Obstet. 2002; 77: 67-75.

3- Roberts JM, Gammill HS. Pre-eclampsia: Recent insights. Hypertension. 2005; 46: 1243-9.

4- Hellgren M. Haemostasis during normal pregnancy and puerperium. Semin Thromb Haemost. 2003; 29: 125-30.

5- Shikany JM, Patterson RE, Agurs-Collins T, Anderson G. Antioxidant supplementation use in women 's health initative participants. Prev Med. 2003; 36: 379-87.

6- Mc Rae KR, Samuls P, Schreiber AD. Pregnancy associated thrombocytopenia, pathogenesis and management. Blood. 1992; 80: 2697-14.

7- Mehta AB, Hoffbrand AV. Pregnancy and haematological effects. In: Postgraduate Haematology. Fifth edition. 2005; P 965.

8- Bick RL. Syndromes of disseminated intravascular coagulation in obstetrics, pregnancy, and gynecology. Objective criteria for diagnosis and management. Hematol Oncol Clin North Am. 2000; 14: 999-1044.

9- May JM, Qu ZC, Juliao S, Cobb CE. Ascorbic acid decreases oxidant stress in indothelial cells caused by the nitroxide tempol. Free Radic Res. 2005; 39: 195-202.

10- Kondo H, TaKahsashi M, Niki E. Peroxynitrite-induced hemolysis of human erythrocyte and its inhibition by antioxidant. FEBS Lett. 1997; 413: 236-8.

11- Sibai BM, Barton JR. Expectant management of severe preeclampsia remote from term: Patient selection, treatment, and delivery indications. Am J Obstet Gynecol. 2007; 196: 514.e1-9.

12- Chappell LC, Seed PT, Kelly FJ, Briley A, Hunt BJ, Charnock-Jones DS, Mallet A, Poston L. Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative stress and placental function. Am J Obstet Gynecol. 2002; 187: 777-84.

13- Thibeault DW. The precarious antioxidant defenses of the preterm infant. Am J Perinatol. 2000; 17: 167-81.

14- Stahl W, Sies H. Antioxidant defense: Vitamins E and C and carotenoids. Diabetes. 1997; 46: 14-8.

15- Turgut M, Basaran O, Cekmen M, Karatas F, Kurt A, Aygun AD. Oxidant and antioxidant levels in preterm newborns with idiopathic hyperbilirubinaemia. J Paediatr Child Health. 2004; 40: 633-7.

16- Dani C, Martelli E, Bertini G. Plasma bilirubin level and oxidative stressing preterm infants. Arch Dis Child Fetal Neonatal Ed. 2003; 88: F119-F23.

17- Poston L, Chappell LC. Is oxidative stress involved in the aetiology of pre-eclampsia? Acta Paediatr Suppl. 2001; 90: 3-5.

18- Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. Proc Soc Exp Biol Med. 1999; 222: 222-35.

19- Raijmakers MT, Dechend R, Poston L. Oxidative stress and preeclampsia: Rationale for antioxidant clinical trials. Hypertension. 2004; 44: 374-80.

20- Zhang C, Williams MA, King IB, Dashow EE, Sorensen TK, Frederick IO. Vitamin C and the risk of preeclampsia results from dietary questionnaire and plasma assay. Epidemiology. 2002; 13: 409-16.

21- Chappell LC, Seed PT, Briley AL, Kelly FJ, Lee R, Hunt BJ, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: A randomised trial. Lancet. 1999; 354: 810-6.

22- Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH. Vitamin C and vitamin E in pregnant women at risk

for pre-eclampsia (VIP trial): Randomised placebo-controlled trial. Lancet. 2006; 367: 1145-54.

23- Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS. Vitamins C and E and the risks of preeclampsia and perinatal complications. N Engl J Med. 2006; 354: 1796-806.

24- Collins K, Blum R. Methods of analysis of vitamins, provitamines and chemical well defined substances having a similar biological effect 1st part, "TEL QUEL". FEFANA Working party. FEFANA, EU Feed Additives and Premixture Association. 2005.

25- Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M. Fetal growth and perinatal viability in California. Obstet Gynecol. 1982, 59: 624-32.

26- Gilbert S. Can vitamins prevent pre-eclampsia. Lancet. 1999; 354: 810-16.

27- Schmidtova M, DubovickyM, Navarova J, Bruckner-ova I, Mach M. Neurobehavioural changes in rats after after neonatal anoxia, effect of antioxidant pretreatment. Neuroendocrinol Lett. 2006; 27 Supplement 2.

28- Reliene R, Schiestl RH. Antioxidants suppress lymphoma and increase longevity in Atm-deficient mice. J Nutr. 2007; 137 (1 Suppl): 229S-32S.

29- Sanodze N, Uberi N, Uberi E, Kulumbegov B. Parameters of oxidative metabolism in neonates suffering from sepsis and anemia. Georgian Med News. 2006; 140: 65-7.

30- Spinnato JA 2nd, Freire S, Pinto E Silva JL, Cunha Rudge MV, Martins-Costa S. Antioxidant therapy to prevent preeclampsia: A randomized controlled trial. Obstet Gynecol. 2007; 110: 1311-8.

31- Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A. Lower rate of preeclampsia after antioxidant supplementation in pregnant women with low antioxidant status. Hypertens Pregnancy. 2006; 25: 241-53.