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Effect of Antioxidants on the Occurrence of Pre-eclampsia in Women at Increased Risk

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Abstract: The objective of this work is: Asses the potential benefit of antioxidant supplementation on the occurrence of preeclampsia at high risk women**. Patients and Methods**: The number of primigravida women's collected was " 294" with gestational age ranged from 18-22 wks. .from (294) prim gravida cases ,(25) women were identified as being at increased risk of pre-eclampsia by abnormal Doppler wave form in either uterine artery at 18-22 weeks' gestation (defined as a resistance index > 95th centile for gestation or the presence of an early diastolic notch) , Another (25) women with history in the preceding pregnancy of pre-eclampsia, eclampsia or HELLP (Hemolysis, elevated liver enzymes, low platelets) . -Total number of eligible participants were, (50) cases. **Design and procedure**: Cases divided randomly into two groups each 25 cases one group received tablets of 1000 mg vitamin C, (Cevitil efferecent. EPICO) + 400 iu of vitamin E (E-Viton 400 pharco). Venous blood for measurement of PAI-1 and PAI-2, The primary outcome measure was the ratio PAI-1 / PAI-2 and the secondary outcome prospectively according to guidelines of international society for the study of hypertension in pregnancy. **Results:** Doppler screening was done on, 294 prim gravidae with gestational age ranged from 18-22 weeks; only 42 women showed abnormal Doppler study so asked to come for rescanning at 24 weeks' gestation. 31 out of 42 patients (75%) have persistent abnormal study. The remainders of the 42 women (25%) were withdrawn from the study at 24 wks.' gestation because they have normal uterine-artery scan. Development of mild preeclampsia was statistically lower ($p<0.5$) in the study than that in the control group. However, development of sever preeclampsia or superimposed preeclampsia was statistically insignificant between both groups. PAI-1 was significantly increased in women developed preeclampsia more than normotensive (167±71.2, 113.8±35.6ng/ml respectively) with P=0.03 IN contrast PAI-2 was significantly decreased in women developed preeclampsia less than normotensive (105±34.9, 181.1±67.9ng/ml respectively) with P=0.018. **Conclusion:** Treatment and prevention trials of preeclampsia have been disappointing to date. However, this study has suggested causal links between oxidative stress and the development of preeclampsia indicate that may be a role for antioxidant vitamins in the prevention of preeclampsia. a high-risk population can be successfully identified on the basis of uterine-artery Doppler screening and pervious history of the disease.

Introduction

Preeclampsia is an important cause of maternal morbidity and mortality (de Swiet M, 2000) and accounts for 5-fold increase in perinatal mortality with iatrogenic prematurity (Farag et al., 2004). Despite the high cost to families and health-service resources, there is no effective management strategy other than

elective delivery, and no therapeutic intervention has been proven to prevent or delay the onset of this disease (Sibai 1998).

This study was primarily designed to assess the potential benefit of antioxidant supplementation on markers of endothelium and placental function. Plasminogen activator inhibitor 1 (PAI-1) is synthesized predominantly by endothelial cells and is a marker of endothelial-cell activation .PAI-1 concentrations increase progressively in the maternal plasma in normal pregnancy and even higher in pre-eclampsia (Halligan et al.,1994) .PAI-2 is synthesized by the placenta ; plasma concentrations also increase progressively in normal pregnancy but decrease with reduced placental function (Halligan et al.,1994) .The ratio of PAI-1 to PAI-2 decrease with reduced placental function (Halligan et al.,1994) .The ratio of PAI-1 toPAI-2 decreases in normal pregnancy as the placental mass increases , but is high in pre-eclampsia owing to endothelial-cell activation and placental insufficiency .Reith and colleagues have suggested that the ratio may be useful as a discriminator between normal and pre-eclampsia pregnancies (Reith et al.,2003) . In the present study it will be used as an index of the disease process for this study. Our secondary outcome measure was the occurrence of pre-eclampsia.

Patients and Methods

This study was carried during the period from October ,2004 to october2005 for pregnant women attending outpatient clinic of obstetric department, Shirbin general hospital –Dakahlia government

Participants: The number of primigravida womens collected was " 294" with gestational age ranged from 18-22 wks. All these patient submitted for Doppler study for the first time, "42" patient showing abnormal Doppler study so asked to come for rescanning at 24 weeks. At " 24 " wks, " 38" pregnant ladies came and "4" patient drop 0ut. The result showing only " 31 " patient with persistent abnormal Doppler study so " 30% " of the 42 women with abnormal waveforms were withdrawn at 24 weeks' gestation because they have normal uterine-artery scan. Further (6) women withdrew from the trial after 24 weeks' gestation :(2) transferred their antenatal care to another area ;(3) didn't want to continue taking tablets throughout pregnancy; and(I) women didn't return for further visits despite several reminders. Another group 28 pregnant women with history of preeclampsia in the previous pregnancy. (3) women of them withdrew from the trial : (I) transferred their antenatal care to another area ; (I) didn't want to continue taking tablets throughout pregnancy ; and (1) women didn't return for further visits despite several reminders .So from (294) prim gravida cases , (25) women were identified as being at increased risk of pre-eclampsia by abnormal Doppler wave form in either uterine artery at 18-22 weeks' gestation (defined as a resistance index > 95th centile for gestation or the presence of an early diastolic notch)

Uterine-artery Doppler screening was done by one observer. Another (25) women with history in the preceding pregnancy of pre-eclampsia, eclampsia or HELLP (Hemolysis, elevated liver enzymes, low platelets). Total number of eligible participants were, (50) cases.

Design and procedure: -

Cases divided randomly into two groups each 25 cases one group received tablets of 1000 mg vitamin C, (Cevitil efferecent. EPICO) + 400 iu of vitamin E (E-Viton 400 pharco). Other group received placebo. We chose to use the combination of vitamins, because vitamin C a water-soluble antioxidant, and vitamin E, a lipid-soluble antioxidant, act synergistically in vitro (Chappell et al., 2002). women (with persistently abnormal wave forms) and those with a previous history of pre-eclampsia were seen every 4 weeks through the rest of pregnancy, these visits were additional to the routine antenatal care arranged for these women. Record of the uterine artery Doppler results was made in the antenatal notes, for all participant in the research study.

At each visit, Venous blood was drawn from an uncuffed arm into tubes containing trisodium citrate (ratio one to nine), for measurement of PAI-1 and PAI-2, the tubes were placed immediately on ice, and centrifuged at 4^0c within 3 hours of sampling. Samples of the supernatant were removed and stored at -70^0c until analysis.

PAI-1 and PAI-2 antigen concentrations were measured by ELISA (provided by Roche Diagnostics Ltd, Bell Lane, Lewes, East Sussex, BN7 1LG, UK). The lower limits of detection were o.5 ug /L (PAI-1) and o.6 ug /L (PAI-2).The primary outcome measure was the ratio PAI-1 / PAI-2 and the secondary outcome prospectively according to guidelines of international society for the study of hypertension in pregnancy, Gestational hypertension was defined as two recordings of diastolic blood pressure of 90 mmHg or higher at least 4 hours apart, and sever gestational hypertension as two recordings of diastolic blood pressure of 110 mmHg or higher at least 4 hours apart or one recording of diastolic blood pressure of at least 120 mmHg . Proteinuria was defined as excretion of 300 mg or more in 24 hours or two readings of 2+ or higher on dipstick analysis of midstream or catheter urine specimens if no 24 hours' collection was available. Women were classified as previously normotensive or with chronic hypertensions before 20 weeks' gestation. for previously normotensive patient , pre-eclampsia was defined as gestational hypertension with proteinuria ,for women with chronic hypertension , superimposed pre-eclampsia was defined by the new development of proteinuria., all women were allocated to an outcome category on the basis of their blood pressure before delivery other adverse perinatal out comes were ; placental abruption (the presence of retro placental clot at delivery and abdominal pain , bleeding , or both immediately before delivery); spontaneous preterm delivery (before 37 weeks gestation) ; intra uterine death , and small for gestational age infants .

statistics

The statistical analysis of data done by using excel program and spss program (statistical package of social science) version 10 on windows 98 on computer compatible with IBM .1st Part was descriptive, in from of mean ± SD and frequency& proportion .2nd part was analytic, for quantitative data (mean ± SD) student t-test was used for comparing betweens 2 groups .For quantitative data (median & range) or frequency & proportion chi-square test was used .P is significant if ≤ 0.05 at confidence interval 95%.

Results

Doppler screening was done on, 294 prim gravidae with gestational age ranged from 18-22 wks.; only 42 women showed abnormal Doppler study so asked to come for rescanning at 24 wks.' gestation. 31 out of 42 patients (75%) have persistent abnormal study. The remainders of the 42 women (25%) were withdrawn from the study at 24 wks.' gestation because they have normal uterine-artery scan. Further 6 women were withdrawn from the study, 2 on request transferred to have their antenatal care at another area, 3 did not want to continue taking medication throughout pregnancy, and only one woman did not return for further visits despite several reminders. The second group of 28 pregnant women with previous history of preeclampsia, 3 of them were withdrawn from the study, one woman on request transferred to have their antenatal care at another area, one did not want to continue taking medication throughout pregnancy, and one women did not return for further visits despite several reminders. So total numbers of eligible participants were (50) cases.

Clinical characteristics of control and study groups

Mean age, body mass index, and blood pressure between study and control groups were statistically insignificant.

Table (1) Baseline characteristics of treated patient cases

Patient profile	Placebo (controlled) (N=25)		Vitamin C+E (N=25)	
	Mean	SD	Mean	SD
Mean (SD) age years	29.8	+/- 5.6	28.9	+/- **6.4**
Mean (SD) body mass index	25.6	+/- 5.6	23.3	+/- **6.0**
Mean (SD) bl.pressuer (mmHg)				
Systolic	110	+/- 12	112	+/- **12**
Diastolic	68	+/- 10	67	+/- **11**
Smokers	**1**		**0**	

Student t-test is used

Percentage incidence of preeclampsia among control and study groups

Development of mild preeclampsia was statistically lower ($p<0.5$) in the study than that in the control group. However, development of sever preeclampsia or superimposed preeclampsia was statistically insignificant between both groups.

Table (2) Percentage incidence of preeclampsia among control and study groups

	Control group N = 25		Study group N =25		X value	P value
	N	%	N	%		
Mild preeclampsia	4	16%	1	4%	X2=1.09	**Ns**
Severe preeclampsia	2	8%	1	4%	X2=0.35	**Ns**
Total with preeclampsia	**6**	**24%**	**2**	**8%**	**X2=2.38**	**Ns**

Percentage incidence of gestational hypertension among control and study groups

Occurrence of gestational hypertension among control group and study group was statistically insignificant.

Table (3) Percentage incidence of gestational hypertension among control and study groups

Percentage incidence gestational hypertension	Control group N = 25		Study group N = 25		X value	P value
	N	%	N	%		
Normal blood pressure	15	60%	18	72%	X2=0.8	**(Ns)**
Gestational hypertension	4	16%	5	20%	X2=0.14	**(Ns)**
Preeclampsia	**6**	**24%**	**2**	**8%**	**X2=2.38**	**(Ns)**

Mean systolic blood pressure value during pregnancy (mmHg)among control and study groups

Mean systolic blood pressure value during pregnancy (mmHg)among control and study groups was statistically insignificant.

Table (4) Mean systolic blood pressure value during pregnancy (mmHg)among control and study groups

groups		Normotensive	Gestational hypertension	Preeclampsia
		N=15 (60%)	N=4 (16%)	**N=6 (24%)**
		Mean ±SD	Mean ± SD	**Mean ± SD**
Systolic	Control group	120 ±12.5	145 ±15.6	**156 ± 3.13**
	Study group	125 ±11.3	150 ±14.6	**160 ±20.3**
	t-test	0.67	0.77	**0.46**
	P value	**0.32**	**0.26**	**0.42**

Mean diastolic blood pressure value during pregnancy (mmHg)among control and study groups

Mean diastolic blood pressure value during pregnancy (mmHg)among control and study groups was statistically insignificant.

Table (5) Mean diastolic blood pressure value during pregnancy (mmHg)among control and study groups

groups		Normotensive	Gestational hypertension	Preeclampsia
		N=18 (72%)	N=5 (20%)	**N=2 (8%)**
		Mean ±SD	Mean ± SD	**Mean ± SD**
Diastolic	Control group	75 ±7.3	100 ±10.2	**105 ±15.7**
	Study group	80 ± 6.5	100 ± 9.6	**110 ±13.7**
	t-test	0.98	0.12	**0.63**
	P value	**0.12**	**0.96**	**0.27**

Mean PAI-1&2 value among control and study groups (abnormal Doppler waveform cases):

PAI-1 was significantly increased in women developed preeclampsia more than normotensive (167±71.2, 113.8±35.6ng/ml respectively) with P=0.03

IN contrast PAI-2 was significantly decreased in women developed preeclampsia less than normotensive (105±34.9, 181.1±67.9ng/ml respectively) with P=0.018.

Table (6) Mean PAI-1&2 value among control and study groups (abnormal Doppler waveform cases)

	PAI-1 Mean ± SD	PAI-2 Mean ± SD
Normotensive	113.8 ± 35.6 ng/ml	**181.1± 67.9 ng/ml**
Preeclampsia	167± 71.2 ng/ml	**105.3 ± 34.9 ng/ml**
t-test	2.31	**2.57**
P value	**0.03***	**0.018***

*** significant**

Discussion

Hypertensive disorders of pregnancy (HDP) constitute the commonest medical disorder diagnosed by obstetricians in clinical practice. It is well recognized that HDP complicate 6-8% of pregnancies (ACOG, 1996). Pre-eclampsia is an important cause of maternal morbidity and mortality (De Sweet., 2003) and account for more than 40% of iatrogenic premature deliveries (Metis et al., 1998). Although there is clear evidence for placental dysfunction and endothelial cell activation in preeclampsia, interest has now turned to the possible factors mediating the interaction between the two oxidative stress has been proposed as providing a link between abnormal placentation and maternal manifestation of preeclampsia (Roberts, Hubel, 2004). There is no widely accepted test for the prediction of pre-eclampsia.

In this study two methods of assessment were used to identify high risk pregnant women for developing pre-eclampsia, the first, prim gravida and The second, uterine artery Doppler waveform analysis provides a surrogate marker of placental perfusion as studied by Ochi et al, (1998). When the normal trophoblastic invasion and modification of spiral arteries is interrupted, there is increased impedance to flow within the uterine arteries and decreased placental perfusion. These pathological process are key features common to the development of pre-eclampsia, and are suspected when the resistance index(RI) fails to decrease in the second trimester, or with the appearance of diastolic notching in the uterine artery waveform (Nicolaides et al; 2002). Papageorghiou et al; 2001 conducted a multicenter cohort study of approximately 8000 unselected singleton pregnancies to determine the utility of transvaginal color Doppler assessment of the uterine artery in the prediction of subsequent development of pre-eclampsia and /or IUGR. Bilateral notching or an elevated PI were considered abnormal finding. The authors concluded that uterine artery Doppler screening at 23 weeks is most informative in identifying the more severely affected fetuses and have a role in routine pregnancy care.

The goal of the study was to determine if prophylactic antioxidants could prevent evidence of endothelial activation, as measured by PAI-1 a marker of endothelial activation and placental dysfunction, as measured by PAI-2. In addition, and even more important from clinical standpoint, the frequency of preeclampsia among control and study cases. It has been conducted on 50 patients divided into two equal groups (control and study groups). PAI-1 is a primary inhibitor of tPA and other plasminogen activators in the blood, PAI -1 limits production of plasmin and serves to keep fibrinolysis in check (Huber, 2001). The ratio of PAI-1/PAI-2 should decreased in normal pregnancy as placental may increase. However, it is high in preeclampsia due to increased endothelial cell activation and placental insufficiency (Granger et al., 2001). In the current study, the level of PAI-1 antigens was significantly higher in preeclampsia than in normal pregnancy (167.7+/- 71.2 versus 113+/- 35.6 ng/ml; $p< 0.05$) in contrast PAI-2 was significantly lower in preeclampsia than in normal pregnancy (105.3+/- 34.9 versus 181 +/- 67.9 mg/ml; $p< 0.001$). This current study nearly equal the same result of Chappell study performed at 1999 showed development of 6 out 25(24%) women in the control group compared with 2 out 25(8%) in study group. This reduction in preeclampsia was mirrored by significant changes in the PAI -1/PAI-2 ratio. The improvement in biochemical function in this small study, might indicate a much larger multicenter study to investigate the outcome. The ratio of PAI-1 to PAI-2 was adopted as the primary outcome measure because it reflects, both endothelial and placental function. The results show clearly that supplementation with vitamins C and E lowered this biochemical indicator of disease in women at risk, thereby supporting the hypothesis of oxidative effect.

In the present study, supplementation throughout the second half of pregnancy with vitamins C and E in women at increased risk of pre-eclampsia had significant beneficial effects on biochemical markers of the disease (plasminogen activator inhibitors -1 and 2 (PAI -1 & PAI -2), and the incidence of preeclampsia decreased in study group compared to control group {2 out 25 (8%)versus 6 out 25(24%)} with insignificant p value. The combination of vitamins C and E used, because vitamin C a water-soluble antioxidant, and vitamin E, a lipid-soluble antioxidant, act synergistically in vitro (Chan, 1993). As regard action of vitamin C as antioxidant understood from that nitric oxide (No), a labile endothelial relaxing factor, is derived from L-arginine by the activity of the enzyme No synthase (Palmer, 1988).

The role of vitamin E in human reproduction has not been investigated in detail. Instead, most investigators have focused on the antioxidant potential of vitamin E and have tried to explain its various action based on this property. Accordingly, it is widely promoted to be helpful in preventing or modulating diseases that are supposedly associated with oxidative stress as hypertensive disorders with pregnancy (Sharma teal; 2006). However, the trial demonstrated that vitamin C and E supplementation was associated with a significantly reduced occurrence of preeclampsia in treated group. In that study analysis those who

completed the study showed a more pronounced effect, with 21 out 81 (26%) women in the control group developing preeclampsia compared with 6 out 79 (8%) in supplemented group.

This current study did not directly address the mechanisms by which vitamins C and E decrease PAI-1/PAI-2 and reduce the risk of pre-eclampsia. Both antioxidants are inhibitors of reactive oxygen species, and a likely explanation of their effect is through this mechanism. Nichikimi, (1975) has been stated that ascorbic acid is a patent scavenger of superoxide radicals. And may thus have helped preserve nitric oxide. In addition, both Alfa-tocopherol and ascorbic acid decrease LDL (low density lipoprotein) oxidation (Fuller et al., 1996)— and ascorbic acid can help to maintain intra cellular glutathione concentration (Toescu et al., 2002). Finally, in this study, supplementation throughout the second half of pregnancy with vitamins C and E in women at increased risk of preeclampsia had significant beneficial effects on biochemical markers of the disease.

Conclusion:

Treatment and prevention trials of preeclampsia have been disappointing to date. However recent studies that have suggested causal links between oxidative stress and the development of preeclampsia indicate that may play a role as antioxidant vitamins in the prevention of preeclampsia.

We concluded there for that a high-risk population can be successfully identified on the basis of uterine-artery Doppler screening and pervious history of the disease. This finding may helpful in the design of future trials of preventive treatment although there is potential for treatment to affect transformation of the uterine-artery Doppler waveform between 20 and 24 wks. of gestation , vitamins C and E are unlikely to have had any effect in our study , given the similar waveform normalization in the placebo and treated groups randomization at 20 wks. rather than 24 wells of gestation ensured that women at increased risk started taking vitamins or placebo at the earliest possible stage of gestation . late initiation of therapy after 24 weeks' gestation has been criticism of some previous trials (Sibai 1998)

because pathophysiological processes are likely to begin many weeks before clinical manifestation of pre-eclampsia.

Although the results of this trial may have substantial implication for the future management of pregnancy multicenter clinical trial with large number of patients is needed before any decisions can be made about clinical management: such atrial should include investigation of optimum dosing and timing of administration, and longer term follow up of infants. Assessment of risk on the basis of uterine-artery Doppler screening may not be feasible in all hospital settings. Any future multicenter trial should also consider the potential use cost-benefit of supplementation with vitamins E and C in all pregnant women. Clinical trial should be undertaking testing the hypothesis that antioxidant vitamins can reduce the risk of preeclampsia, reduce maternal and fetal mortality rates, and increase birth weight.

such clinical trials also should incorporate study of biomarker and other measures of risk factors and determine predictors and early pathophysiological changes of preeclampsia in low-risk and high-risk populations. This study, although encouraging, was quite small and thus the efficacy and safety of antioxidant treatment for the infant require confirmation in larger studies. as a significant reduction in the proportion of women with preeclampsia.

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