The Possible beneficial effects of Antioxidant drugs (Vitamin C and E) and Allopurinol in the management of Pre-eclamptic patients treated with Methyldopa.

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Summary:

Background: Pre-eclampsia is culmination of a multi-step process that related in part to elevated oxidative stress and associated with hyperurecimia.

Patients and methods: Thirty normotensive and hundred pre-eclamptic pregnant women attending to Al-Basra hospital of pediatrics, obstetrics and gynecology were participated in this study. The patients were randomized into six groups. They were treated with methyldopa alone, methyldopa plus vitamin C, methyldopa plus vitamin E, methyldopa plus vitamin C and E, and methyldopa plus allopurinol. The oxidative stress (MDA), renal function parameters, systolic and diastolic blood pressure were evaluated before treatment and 14 day after initiation of therapy.

Results: Using allopurinol, vitamin C, vitamin E, and a combination of vitamin C and E together with methyldopa in pre-eclampsia can produce a significant reduction in the level of oxidative stress, on the other hand, some of these supplemental antioxidants can produce a significant reduction in systolic and diastolic blood pressures, serum creatinine and proteinurea as well as serum uric acid concentration in different extent.

Conclusion: Antioxidants and allopurinol when co-administrated with methyldopa, improves the maternal and biochemical indicators of pre-eclampsia and produce a better control of blood pressure.

Keywords: Pre-eclampsia, Antioxidants, Oxidative stress, Vitamin C, Vitamin E, Allopurinol.

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Introduction:

Hypertensive disorders of pregnancy are considered as a major cause of maternal and perinatal mortality and morbidity worldwide, particularly in developing countries. Pre-eclampsia is a syndrome unique to pregnancy characterized by the new onset of hypertension, proteinurea and edema in the latter half of gestation. Several studies have shown that elevated oxidative stress might implicate in the pathophysiology of pre-eclampsia. In the early stages of pre-eclamptic pregnancy, failure of trophoblast invasion of the spiral arteries leads to reduced placental perfusion, and hypoxia. The hypoxia/reperfusion injury leads to increased expression of xanthine oxidase and NADP (H) oxidase and resultant increased generation of reactive oxygen species and peroxynitrite , which is followed by activation of the maternal endothelium, leading to the multisystem disorders seen in severe pre-eclampsia (1,2). Xanthine oxidase, an enzyme which produces uric acid spontaneously, is an important major source for oxygen free radical production within theendothelium. (Vitamin E), Antioxidants, including the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, as well as, non-enzymatic compounds such as α-tocopherol β-carotene, ascorbate (vitamin C), and glutathione can all counteracting free radicals damage (3)**.** Vitamin C, a water-soluble antioxidant, is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids. Vitamin E is a fat-soluble vitamin widely distributed in the membranes of cells, has generally been considered as an excellent antioxidant that protects cell membranes and other fat-soluble parts of the body from damage by free radicals. Vitamin C works along with vitamin E and the enzyme glutathione peroxidase to stop free radical chain reactions and since ascorbic acid is water soluble; it can work both inside and outside the cells to combat free radical damage (4)**.** Allopurinol is a purine analog, it reduces the production of uric acid and inhibits the oxygen free radical production within the endothelium by competitively inhibiting the last two steps in uric acid biosynthesis that are catalyzed by xanthine oxidase (5) **.**

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Subjects and methods:

This study was carried out on 130 women with age ranged between (20-42) years (mean 30.99 ± 0.47), while they were attending Al-Basra Hospital of Pediatrics, Obstetrics and Gynecology from September 2007 to September 2008. They were divided into: 30 subjects are apparently healthy and are served as control (group1). The 100 patients

with pre-eclampsia were allocated into: 20 patients treated with 250 mg methyldopa three times daily (Group 2). 20 patients treated with 250 mg methyldopa three times daily plus 500mg of vitamin C twice daily (Group 3). 20 patients treated with 250 mg methyldopa three times daily plus 400 IU of vitamin E daily (Group 4). 20 patients treated with 250 mg methyldopa three times daily plus 500 mg of vitamin C twice daily and 400 IU of vitamin E daily (Group 5), and 20 patients treated with 250 mg methyldopa three times daily plus 300mg allopurinol daily (Group 6). Venous blood was collected from the forearm from all subjects participated in this study, by vein puncture before starting therapy, and after 14 days of initiation of therapy. Each sample placed in EDTA-free plastic tube and left at room temperature for 30 minutes for complete clotting of blood, then centrifuged at 3000 rpm for 10 minutes. Serum obtained was divided into: 0.5 ml of fresh serum for measurement of MDA within 24 hour and the rest part of serum was freezed at (–20) C° to be stored until time of assay of other parameters.Serum MDA levels, the end product of lipid peroxidation were estimated by the method of Beuge et al. (6) Colorimetric determination of serum uric acid concentration is based on the method described by Fossati (7) and the measurement of serum creatinine concentration has been done by the method described by Henry RJ (8) The determination of proteinurea concentration based on the phenomenon called the "Protein Error of Indicators" which depends on ability of protein to alter the color of some acid-base indicators without altering the pH (9)**.** Statistical analyses were performed using Student T-test and ANOVA test to examine the degree of significance (P <0.05 was considered as significant).

Results:

Serum MDA levels were significantly higher in patients with pre-eclampsia before starting supplementation when compared to normal control group. These levels were significantly reduced (15.46%, 22 %, and 17.89%) after 14 day supplementation with vitamin C, combination of vitamin C and E, and allopurinol respectively, while serum MDA level was elevated (5.69%) after 14 days methyldopa therapy and did not significantly reduced (7.94%) after 14 days vitamin E supplementation when compared to baseline serum MDA values (p>0.05) as showed in table (1). Systolic and diastolic blood pressures were significantly reduced (6.11%, 3.1%) (4.96%, 4.12%) after 14 day supplementation with combination of vitamin C and E, and allopurinol respectively, while the systolic and diastolic blood pressures were not significantly reduced (0.52%, 2.25%, and 0.68%), (1.8%, 1.59%, and 1.82%) after 14 day therapy with methyldopa only, or supplementation with vitamin C, and vitamin E respectively as compared to the baseline systolic and diastolic blood pressure (p>0.05). Table (2).

Table (1): The effect of treatment with methyldopa only (group 2), or methyldopa and one of the following: vitamin C (group 3), vitamin E (group 4), combination of vitamin C and E (group 5), and allopurinol (group 6) on

Group	Number of patients	Baseline serum MDA (µmol/l)	Serum MDA after 14 days of treatment (µmol/l)
Group1	30	0.67± 0.04	-
Group 2	20	2.11 ± 0.12†	2.23 ± 0.95
Group 3	20	1.94 ± 0.11†	1.64 ± 0.09*
Group 4	20	2.14 ± 0.08†	1.97 ± 0.09
Group 5	20	2.00 ± 0.10†	1.56 ± 0.11*
Group 6	20	2.18 ± 0.09†	1.79 ± 0.09*

serum MDA level in patients with pre-eclampsia.

† Significant at p<0.05 as compared with control group.

* Significant at p<0.05 as compared with baseline values. Values are presented as mean ± SEM.

Table (2): The effect of treatment with methyldopa only (group 2), or methyldopa and one of the following: vitamin C (group 3), vitamin E (group 4), combination of vitamin C and E (group 5), and allopurinol (group 6) on the systolic and diastolic blood pressure in patients with pre-eclampsia.

Group	No. of patients	Baseline SBP mmHg	SBP after 14 days of treatment	Baseline DBP mmHg	DBP after 14 days of treatment
Group 1	30	123 ± 0.59	-	78.17 ± 1.1	-
Group 2	20	144.5 ± 1.02†	143.75 ± 0.95	96 ± 1.61†	94.25 ± 1.4
Group 3	20	144.25 ± 1.22†	141 ± 1.65	94.25 ± 1.16†	92.75 ± 1.17
Group 4	20	147 ± 1.11†	146 ± 1.24	96.25 ± 1.66†	94.5 ± 1.58
Group 5	20	147.25 ± 1.17†	138.25 ± 1.16*	95.75 ± 1.46†	91 ± 1.42*
Group 6	20	145 ± 1.03†	140.5 ± 1.66*	97 ± 1.72†	93 ± 1.1*

† Significant at p<0.05 as compared with control group.

* Significant at p<0.05 as compared with baseline values. Values are presented as mean ± SEM.

Serum creatinine and proteinurea concentrations were significantly reduced (22.99%, 23.33%, 25.15%, and 50.00 %) (46.15%, 36.11%, 51.28%, and 56.1%) after 14 days of supplementation with vitamin C, vitamin E, combination of vitamin C and E, and allopurinol respectively, while the serum creatinine and proteinurea concentrations were elevated (4.92%), (7.5%) after 14 days of treatment with methyldopa only when compared to baseline concentration (p>0.05) as mentioned in table (3)

Table (3): The effect of treatment with methyldopa only (group 2), or methyldopa and one of the following: vitamin C (group 3), vitamin E (group 4), combination of vitamin C and E (group 5), and allopurinol (group 6) on the renal function parameters (serum creatinine, and proteinurea) in patients with pre-eclampsia.

Group	No. of patients	Baseline serum creatinine conc. (mg/dl)	serum creatinine conc. after 14 days of treatment (mg/dl)	Baseline protein urea conc.	Proteinurea conc. after 14 days of treatment
Group 1	30	0.64 ± 0.03	-	0	-
Group 2	20	1.83 ± 0.16†	1.92 ± 0.09	2 ± 0.16†	2.15 ± 0.15
Group 3	20	1.74 ± 0.12†	1.34 ± 0.08*	1.95 ± 0.18†	1.05 ± 0.18*
Group 4	20	1.8 ± 0.13†	1.38 ± 0.08*	1.8 ± 0.17†	1.15 ± 0.21*
Group 5	20	1.71 ± 0.12†	1.28 ± 0.1*	1.95 ± 0.18†	0.95 ± 0.18*
Group 6	20	1.76 ± 0.13†	0.88 ± 0.04*	2.05 ± 0.18†	0.9 ± 0.16*

† Significant at $p<0.05$ as compared with control group.

* Significant at $p<0.05$ as compared with baseline values. Values are presented as mean ± SEM.

Table (4) showed that serum uric acid concentration was significantly ($p<0.05$) reduced (18.42% and 18.67%) after 14 day supplementation with combination of vitamin C and E, and allopurinol respectively, while the serum uric acid concentration was not significantly reduced (0.33%, 10.53%, and 3.56%) after 14 days of treatment with methyldopa alone, supplementation vitamin C, and vitamin E respectively when compared to baseline serum uric acid concentration ($p>0.05$).

Table (4): The effect of treatment with methyldopa only (group 2), or methyldopa and one of the following: vitamin C (group 3), vitamin E (group 4), combination of vitamin C and E (group 5), and allopurinol (group 6) on the serum uric acid concentration in patients with pre-eclampsia.

Group	No. of patients	Baseline serum uric acid conc. (mg/dl)	serum uric acid conc.after 14 days of treatment (mg/dl)
Group 1	30	4.58 ± 0.3	-
Group 2	20	6.01 ± 0.34†	5.99 ± 0.34
Group 3	20	5.32 ± 0.32†	4.76 ± 0.28
Group 4	20	5.34 ± 0.31†	5.15 ± 0.27
Group 5	20	6.08 ± 0.28†	4.96 ± 0.26*
Group 6	20	5.73 ± 0.32†	4.66 ± 0.3*

† Significant at $p<0.05$ as compared with control group.

* Significant at $p<0.05$ as compared with baseline values. Values are presented as mean ± SEM.

Discussion:

The exact etiology of pre-eclampsia is not established yet, and maternal symptoms are thought to be secondary to endothelial cell dysfunction. It has been suggested that free radicals are likely promoters of maternal vascular dysfunction. In pre-eclampsia, there is an imbalance between lipid peroxidation and antioxidant defense mechanism, leading to endothelial dysfunction and free radical-mediated endothelial cell injury (10)**.**This imbalance is either due to increased antioxidants consumption or due to increased production of free radicals by the placenta (11)**.**The results obtained in this study show that circulating levels of oxidative stress marker (MDA) before starting therapy was significantly higher in patients with pre-eclampsia (group 2, 3, 4, 5 and 6) when compared to normal control (group 1) ($p<0.05$), table (1). These results were consistent with those reported by Maarten et al (2008) (12) and Mehendale et al. (2008) (13), who have found significant increase in plasma MDA levels in pre-eclamptic patients when compared to normal pregnant women. Therapy with combination of vitamin C and E was found to reduce serum MDA concentration to greater extent than therapy with vitamin C alone, this could be explained by the fact that vitamins C and vitamin E are potent inhibitors of reactive oxygen species, acting synergistically to prevent lipid peroxidation. Vitamin C also scavenges reactive oxygen species (ROS) in the aqueous phase and recycles lipid-soluble vitamin E to combat ROS-induced tissue damage (14). Therefore supplementation with vitamin C and E may have beneficial effects in pre-eclampsia. These effects are related to their ability to reduce oxidative stress or halting endothelial damage, and thus the development of pre-eclampsia. All these effects may result in decrease in the multisystem disorders seen in pre-eclampsia and prevention of development of severe pre-eclampsia (15). These results were consistent with those reported by Chappell et al. (2002) (16), who have reported, that the preventive intense of the administration of the anti-oxidant vitamins C and E to members of a group at risk of developing pre-eclampsia was effective in the reduction of the incidence of pre-eclampsia in this group. In contrast to vitamin C, vitamin E therapy was slightly, but not significantly reduced serum MDA; this may be due to that vitamin E is located inside the cell membrane lipids; it is a principal antioxidant in breaking the chain reaction of lipid peroxidation. The addition of antioxidants to methyldopa was found to reduce systolic and diastolic blood pressures, table (2). Vitamin C is potent scavenger of free radicals, that may serve to prevent oxidation of nitric oxide (NO) (endothelium dependent vasodilator) to maintain the vasodilatation of blood vessels and thereby reducing blood pressure. Vitamin C is important for preservation of tetrahydrobiopterin (BH_4), an important cofactor of nitric oxide synthase (NOS). Vitamin C is also

necessary for release of NO from S-nitrosothiols, particularly S-nitrosoalbumin, which constitute an important circulating reservoir of releasable NO. Effective release of NO from albumin requires that adequate amounts of vitamin C exist in the circulation. In women with pre-eclampsia, the release of NO is inhibited by the observed decreases in plasma vitamin C. Vitamin C can improve nitric oxide activity by scavenging superoxide radicals and thereby reducing blood pressure(17). Vitamin C also can help in maintain intracellular glutathione concentrations that has benefit in reducing free radicals and so reducing oxidative stress and MDA concentration(18). In addition, vitamin C may also increase prostacyclin production and lower blood pressure by decreasing oxidative stress and up-regulation of glutathione peroxidase(18).Renal function parameters: serum creatinine and proteinurea concentrations were significantly reduced when vitamin C, vitamin E, and combination of vitamin C and E were added to methyldopa treatment, table (3). Considerable experimental evidence supports the view that reactive oxygen species (ROS) play a key role in the pathophysiologic processes of renal diseases. Various stimuli, including ROS, have been identified as promoters of inflammatory responses occurring in glomerular cells (19). The antioxidants, such as vitamin E and vitamin C, may play a key role against the glomerular inflammatory processes, through a diminution of the activity of inflammatory enzymes and cytokine secretion, or by inhibiting the activity of nuclear factor-kappa B (NF-kB) (20). These results were consistent with Lee et al. (1997) (21), and Ricardo et al. (1994) (22), who have been observed that several antioxidant agents such as vitamin E (21), and vitamin E/ascorbic acid (22) are able to ameliorate or prevent induced renal damage.However, serum uric acid concentration was significantly slightly reduce when patients supplied with combination of vitamin C and E and slightly reduced when supplied with vitamin C and vitamin E, table (4). Studies found higher plasma uric acid concentrations in pre-eclampsia than in normotensive pregnancy. Uric acid has pro-inflammatory and vasoconstrictive mechanisms, which would appear to be counterintuitive. Uric acid may also function as an antioxidant and scavenger of peroxynitrite (23). However, when uric acid acts as an antioxidant, it will give up an electron to become a urate radical, which is in itself a weak oxidant. The urate radical rapidly reacts with substances such as ascorbate, resulting in the regeneration of uric acid. However, if ascorbate concentrations are low, as observed in pre-eclampsia, the urate radical could theoretically accumulate; with pro-oxidant effects. Regarding therapy with allopurinol there was significant ($p<0.05$) reduction in serum MDA level, systolic blood pressure, and diastolic blood pressure, table (1, 2). Our results were consistent with that reported by George et al. (2006) (24), who have showed that a steep dose-response relationship exists between allopurinol and its effect on endothelial function. The heme–heme oxygenase (HO) system is a regulator of endothelial cell integrity and oxidative stress. HO catalyzes the oxidative cleavage of heme group to biliverdin, carbon monoxide, and iron. During heme oxygenase enzyme reaction, the concentration of toxic heme is reduced and equimolar concentrations of the antioxidant bilirubin as well as carbon monoxide are produced, the latter being a potent relaxant of vascular smooth muscle. Induction of heme oxygenase activity, as an additional beneficial effect of allopurinol. HO-1 gene up-regulation has also been shown to attenuate Ang II-mediated DNA damage in endothelial and kidney cells (25). Carbon monoxide, in addition to activating the cyclic guanosine monophosphate (cGMP) pathway and eliciting vasodilatation, has also been shown to function as an anti-inflammatory and cytoprotective molecule in the renal tissues. Allopurinol was also significantly reducing renal function parameters: serum creatinine and proteinurea concentrations as well as significantly reducing serum uric acid after 14 days of treatment, table (3, 4). Tàlosi et al. (2001) (26), reported that oral supplementation with allopurinol could have a beneficial effects on experimental pregnancy-induced pre-eclampsia-like disease in animals, and could postponed the occurrence of symptoms of pre-eclampsia in animal model, and have found that mean arterial blood pressure, and kidney function parameters (serum creatinine, and proteinurea) and serum uric acid in allopurinol treated group were significantly lowered than that in non-treated group.

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