

Effect of Vitamin C ,Folic acid and Iron Supplements on Oxidative Stress in Preeclamptic Women in Hilla City

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Abstract:

Pre-eclampsia is a disease characterized by elevation of blood pressure and proteinuria after the 20th week of gestation. Some supplements are used during pregnancy like vitamin C , folic acid and iron can effect on oxidative stress matter in preeclamptic women and may act as antioxidant . The aim is to demonstrate the effect of vitamin C , folic acid and iron supplements on the values of reduced glutathione and malondialdehyde (oxidative stress markers) in preeclamptic, non-anemic pregnant women. The results showed that vitamin C and folic acid reduce the oxidative stress but iron supplements elevate it. Also the results revealed that the larger dose and early starting of vitamin C reduced the oxidative stress more than lower dose and late starting of it but there is no significant differences when using different doses and different time of starting of folic acid , while the larger dose and early starting of iron supplements elevate the oxidative stress more than lower dose and late starting of it. It can be concluded that vitamin C and folic acid are beneficial but there is no need to use iron supplements in non anemic preeclamptic women.

Key words : Vitamin C, folic acid , iron supplements & oxidative stress.

الخلاصة

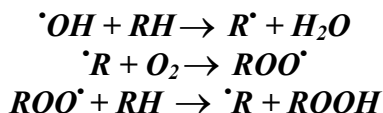
مرض قبل الشنبح يتميز بارتفاع في ضغط الدم والبروتين البولي بعد الاسبوع العشرين من الحمل . بعض المكملات الغذائية التي استخدمت خلال فترة الحمل مثل فيتامين C وحمض الفوليك والحديد له تأثير على حالة الاجهاد التأكسدي في النساء اللواتي يعانين من هذه المتلازمة وقد تعمل كمضادات للأكسدة. الهدف من هذه الدراسة هو التعرف على تأثير فيتامين C وحمض الفوليك ومكملات الحديد على قيم الكلوتاثيون المختزل و المالونديالدهيد (علامات الاكسدة) عند النساء الحوامل المصابات بمرض قبل الشنبح و الغير المصابات بفقر الدم . أظهرت النتائج أن فيتامين C وحمض الفوليك يقلل من الاجهاد التأكسدي بينما تناول الحديد تزيده. ايضا كشفت النتائج أن الجرعة الكبيرة والبدء في وقت مبكر من فيتامين C يخفض الاجهاد التأكسدي أكثر من الجرعة القليلة والبدء في وقت متأخر من ذلك، ولكن ليس هناك فروق ذات دلالة إحصائية عند استخدام جرعات مختلفة ووقت بدء الاستخدام لحمض الفوليك، من جهة أخرى الجرعة الكبيرة والبدء في وقت مبكر باستخدام الحديد تزيد حالة الاكسدة أكثر من الجرعة القليلة والبدء في وقت متأخر من ذلك. يمكن أن نستنتج أن فيتامين C وحمض الفوليك مفيد في تقليل الاجهاد التأكسدي وليس هناك حاجة لاستخدام الحديد لدى النساء المصابات بسندمية الحمل وغير مصابات بفقر الدم.

الكلمات المفتاحية : فيتامين C ، حمض الفوليك ،مكملات الحديد و الاجهاد التأكسدي

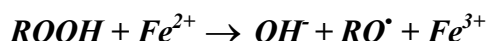
Introduction:

Pregnancy is a developmental crisis in a woman's life. Changes in many of the body's biochemical function during pregnancy leads to a high demand for energy and an increased oxygen requirement. This leads to increase utilization of oxygen, resulting in consequent acceleration in the production of reactive oxygen species (ROS) and increased levels of free radicals and oxidative stress (Bray JJ, *et al* 1999). and its complications especially pre-eclampsia which is a human pregnancy-specific disorder that adversely affects the mother (by vascular dysfunction) and the fetus (by intrauterine growth restriction), it is characterized by vasospasm, increased peripheral vascular resistance, and thus reduced organ perfusion . Pre-eclampsia is diagnosed by the new development of hypertension (usually $\geq 140/90$ mmHg) occurring after week 20 of gestation with significant proteinuria (either ≥ 300 mg protein per day or an urinary protein/creatinine ratio ≥ 30 mg/mmol) and remission of these signs after delivery. Preeclampsia is the leading cause of maternal mortality in the developed

countries (Lindheimer MD, *et al* 1999). NAD(P)H oxidases are a major source of superoxide in neutrophils and vascular endothelial cells and have also been reported in human trophoblast (Manes C 2001). Potential stimuli for the activation of NAD(P)H oxidase in pre-eclampsia include raised feto-placental vascular stress, elevation of maternal plasma cytokine concentrations, and enhanced angiotensin II (Ang II) sensitivity. Thus there is considerable evidence to implicate activation of NAD(P)H oxidase in placental oxidative stress associated with pre-eclampsia (Brown MA, *et al* 1997). The reactions of oxygen free radicals with polyunsaturated lipids have been extensively researched. The peroxidation reactions differ among fatty acids (RH) depending on the number and position of the double bonds on the acyl chain (Frankel, E.N 1985).



The lipid hydroperoxide (ROOH) is unstable in the presence of Fe or other metal catalysts because ROOH will participate in a Fenton reaction leading to the formation of reactive alkoxy radicals:



Therefore, in the presence of Fe, the chain reactions are not only propagated but amplified. Among the degradation products of ROOH are aldehydes, such as malondialdehyde, and hydrocarbons. Malondialdehyde (MDA) production is recognized as a marker for lipid peroxide and as an end-product of peroxidation (Slater TF 1984). Reduced glutathione or GSH, is a relatively small molecule ubiquitous in living systems. GSH is the smallest intracellular thiol (SH) molecule. Its high electron-donating capacity (high negative redox potential) combined with high intracellular concentration generate great reducing power. This characteristic underlies its potent antioxidant action and enzyme cofactor properties. The reducing power of ascorbate helps conserve systemic GSH (Knapen MFCM 2000).

L-ascorbic acid (vitamin C) acts as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress. It can directly scavenge oxygen free radicals with and without enzyme catalysts and can indirectly scavenge them by recycling tocopherol to the reduced form. There are many studies implied that vitamin C act as a prophylactic agent to prevent or decrease oxidative stress in pregnant women (Beazley D, *et al* 2005), (Alice R. *et al* 2006).

Folic acid supplements is prescribed for most pregnant women especially in the first trimester to prevent genetic or DNA disorders. Recent studies have shown an association between hyperhomocysteinemia and both gestational hypertension and pre-eclampsia (Roberts JM, *et al* 2001), (Vollset SE, Refsum H, *et al* 2000). It is hypothesized that hyperhomocysteinemia might damage the vascular endothelium of the developing placenta by promoting oxidative stress, thereby increasing contractile response and the production of procoagulants and vasoconstrictors and it has been shown that folic acid supplementation decreases plasma homocysteine concentrations, so decreases oxidative stress during pregnancy (Sonia Hernández-Díaz, *et al* 2002).

Many gynecologist in developing countries are prescribing iron supplements for pregnant women without considering the Hb test and different national and international organizations recommend daily antenatal iron supplementation generally associated with folic acid and often with other micronutrients (WHO 2006).

One of research groups has made recommendations related to the dangers of high iron doses. The exact mechanisms for this increased risk may be due to excessive free radicals early and during pregnancy appear critical in modifying placentation and

normal fetal development (Biondi C , *et al* 2005), (Lunghi L, *et al* 2007), (King SM, *et al* 2008). Poor placental perfusion in hemoconcentrated states because of increased blood viscosity, has also been proposed (Stepan H, *et al* 2004).

Aim of the study: To demonstrate the effect of Vitamin C , folic acid and iron supplements on the values of GSH, MDA (oxidative stress markers) in preeclamptic, non-anemic pregnant women .

Subjects and methods:

Study samples of 75 preeclamptic and non anemic pregnant women with an age range of 20-30 years were collected from Babylon Hospital for Pediatric and Maternity , in Hilla city how visited the counseling department and the supplements were prescribed under supervision of different physicians specialists in gynecology according to the duty . The preeclamptic pregnant women were divided into 3 groups according to the type of supplements were taken, 25 with vitamin C supplements , 25 with folic acid supplements and the last 25 group with iron supplements. Each group subdivided into 2 groups according to dose taken and another subdivision according to time of starting supplements ; early starters from the first trimester and continuous, and late starters in third trimester. To compare the results, 20 preeclamptic, non anemic and age matched pregnant women without using such supplements, were investigated. A questionnaire was designed which contained the age, gestational age, presence of other diseases and complications, whether the subjects were taking other medications or not, the type and the dose of supplements and the gestational week of starting these supplement. Serum reduced glutathione (GSH) was determined by using DTNB (5,5-dithiobis(2-nitrobenzoic acid)) which is a disulfide chromogen that is readily reduced by sulfahydryl group of GSH to an intensely yellow compound .The absorbance of the reduced chromogen is measured at 412 nm directly proportional to the GSH concentration (Burtis CA, *et al* 1999).

Serum malondialdehyde (MDA) level was determined by the test based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA₂ product that absorb strongly at 532 nm (Raad K. Muslih, *et al* 2002).

Biostatistical analysis:

The results were expressed as Mean \pm SD. Student's t-test was used to verify the association of oxidative stress parameters (GSH and MDA) with type of supplements were taken by preeclamptic women. Significant variation was considered when P-value was less than 0.05.

This analysis was carried out by using the SPSS programme.

Results and discussion :

Measurement of reduced glutathione (GSH) and malondialdehyde (MDA) in preeclamptic pregnant women :

Reduced glutathione (GSH) and malondialdehyde (MDA) levels were measured in sera of all subjects included in the study (3 groups of subjects used different type of supplements and of 20 preeclamptic women without any supplements which is considered as control group) .The results were analyzed using student's t-test. There was significant ($p<0.001$) increase of GSH values and significant decrease ($p<0.005$) in MDA levels in sera of group of preeclamptic women used vitamin C when compared with those of the control group. For preeclamptic women used folic acid, the results showed significant ($p<0.01$) increase of GSH values and non significant difference in MDA levels while for those who used iron supplements, the results indicated a non significant difference of GSH values and significant increase ($p<0.05$) in MDA levels when compared with those of the control group (Table1).

Study showed that the oxidative stress was less in the vitamin C group than in the control group and this finding is corresponding with the findings in an earlier trial that supplementation with vitamins C and E was beneficial in reducing oxidative stress in women at high risk of preeclampsia (Beazley D, *et al* 2005).

Preeclampsia remains a frequent and potentially dangerous complication of pregnancy. The placenta appears to be the principal source of free radical synthesis but maternal leukocytes and the maternal endothelium are also likely contributors. Several reports have suggested an important role for placental trophoblast NAD(P)H oxidase in free radical generation in preeclampsia (Redman CWG, *et al* 2000). Increased expression of NAD(P)H oxidase subunits in both trophoblast and placental vascular smooth muscle cells in placental tissue of women with preeclampsia has been reported (Dechend R., *et al* 2003). Some antioxidants like vitamin C, which has a great reducing power, not only detoxify free radicals but also are involved in redox-sensitive gene expression and inhibition of apoptosis. Importantly, vitamin C directly inhibits monocyte NAD(P)H oxidase activity, thereby preventing phosphorylation (Rossig L, *et al* 2001). Vitamin C might be expected to inhibit the vascular and placental isoforms. This multimode of action may be fortuitous in relation to preeclampsia prevention, because vitamin C may not only inhibit the lipid peroxidation chain reaction but also could minimize the excessive generation of free radicals by inhibition of NAD(P)H oxidase in both placental tissue and maternal neutrophils, reduce placental apoptosis, and inhibit leukocyte and endothelial cell activation (Zhang C, *et al* 2002).

There are several lines of biologic evidence that tend to support the role of folic acid in reducing oxidative stress in pregnancy and its complication by reducing homocysteine levels (Vollset SE, *et al* 2000), (Sanchez SE, *et al* 2000). On the other hand, folic acid supplementation improves endothelial function in adults with hyperhomocysteinemia which eventually result in decrease of oxidative stress (Woo KS, *et al* (2002). Results from previous observational data have suggested that folic acid might reduce the risk of various cardiovascular diseases and some other diseases beside reducing oxidative stress (Rimm EB, *et al* 1998) in which similar to the present observations.

Regarding results of group used iron supplements which implied an increase in oxidative stress when compared with control group, can be related to the reaction of Fe^{2+} with unsaturated fatty acids in the presence of O_2 which initiate a lipid peroxidation cascade in biological membranes and lipoproteins due to the production of highly reactive OH^\cdot (Casanueva E, *et al* 2003). Therefore transition-metal-based radicals may be responsible for initiating the peroxidation of lipids and the formation of conjugated diene double bonds, fluorescent chromolipids and alkoxyl and peroxy radicals that can propagate lipid peroxidation to final end products such as pentane, ethane and malondialdehyde (Benzie, I. F 1996). The oxidative stress and in vivo consequences caused by reactive iron species (mostly free iron) have been studied in detail. Many of the deleterious effects of hemochromatosis and other chronic iron-overload conditions, some cancers, aging, neurodegenerative diseases and atherosclerosis are attributed to or contributed by them. ROS damage is observed in and explains many of the effects secondary to temporary iron excess as in acute iron intoxication, hemolytic episodes and reperfusion injury (Lauffer R. B 1992).

Although World Health Organization (WHO) has made its recommendations, there is no worldwide consensus concerning iron prophylaxis and therapy during pregnancy. There does exist a contradiction between scientific results and common practice. Most gynecologists reach for iron tablets when the hemoglobin value drops

below normal range. In most cases even serum ferritin content or MCV value are not essential in decision-making. There is no conclusive evidence that improvement of the iron status during the first and second trimester would improve the outcome of pregnancy and the hemoglobin values in the third trimester are not considered to influence the outcome significantly at all. From the point of view of oxidative stress, ferrous iron (that is traditionally the form of treatment) is a potent pro-oxidant (Aune Rehema, *et al* 2004).

Effect of dose of Vitamin C supplements and duration of using it on oxidative stress markers :

The results revealed that there was a significant ($p<0.05$) increase of GSH values in sera of preeclamptic women who started using vitamin C early in comparison with those who started using it late and a non significant differences related to vitamin C dose.

Regarding MDA levels, there was a significant decrease ($p<0.05$) in the sera of women used vitamin C in larger dose when compared with those used a lower dose and a significant decrease ($p<0.05$) in the subjects who started using it early when compared with those used it at the late stage (table 2)

Vitamine C is a powerful antioxidant agent act by direct scavenging free radicals or indirect way and so when increase the level of ascorbic acid in body to appropriate limit will increase its antioxidant activity . In previous study where groups of animals fed the high ascorbate dose had significantly lower oxidative stress markers associated with lipid damage like F2-isoprostanes and its elevation have been detected under conditions of increased oxidative stress , and administration of antioxidants, in particular ascorbate and α -tocopherol, has been shown to reduce their levels (Kent Chen1, *et al* 2000).

A wealth of previous experimental and epidemiological data suggests that excess LDL oxidation may be partly responsible for the development of atherosclerosis. Because vitamin E and C inhibit LDL oxidation *ex vivo*, a logical strategy to reduce oxidative stress may include the administration of antioxidants such as vitamin E and C . large pharmacological doses of vitamin C improve endothelial dysfunction in subjects with diabetes and hypertension, and long term vitamin C therapy was found to improve endothelial function in children with hyperlipidaemia (Kwang Kon Koh, *et al* 2009).

Effect of dose of Folic acid supplements and its duration of its use on oxidative stress markers :

No statistically significant correlations between individual parameters were found in using different doses or different onset of starting folic acid supplements (Table 3) The effect of folic acid supplementation might be difficult to identify once populations have been exposed to foods fortified with folate. But the results obtained from present study are in contrast with the results of other study which found that early folic acid supplementation during pregnancy was associated with a lower risk of preeclampsia and its complication more than late supplements (Sonia Hernández-Díaz, *et al* 2002).

Effect of dose of Iron supplements and duration of its use on oxidative stress markers:

The study implied that there was non significant differences of GSH values in using different doses of iron but these values decreased significantly ($p<0.05$) in preeclamptic women who early starting iron supplements when compared with subjects who started using it on a late stage.

For MDA levels there was a significant increase ($p < 0.05$) with larger dose and early starting the supplements when compared with lower dose and late starting of iron supplements respectively (Table 4).

These results revealed that as the dose and duration of using iron supplements increase in non anemic women, the oxidative stress will increase by increasing lipid peroxidation. In agreement with previous study which stated that subjects using iron supplements on a daily bases will have significantly higher plasma ferritin than weekly bases. Oxidative stress may be involved in these results: high Hb levels can reduce placental perfusion and high ferritin levels postpartum may reflect temporary iron excess (Casanueva E, *et al* 2003). High serum ferritin late in pregnancy, possibly associated with excess iron and/or inflammation (Scholl TO 1998). Clinical studies have made recommendations related to the dangers of high iron doses may be due to poor placental perfusion in hemoconcentrated states because of increased blood viscosity (Stepan H, *et al* 2004). Other research considered that most probably the elevated plasma thio-barbituric-acid-reacting-substances (TBARS) concentrations reflect iron-induced toxicity manifested by lipid peroxidation (and oxidative stress) in which TBARS levels rose so dramatically when 60 mg of iron, were consumed daily in contrast to twice the doses of such supplements consumed only once weekly make these findings significant as possible indicators of iron toxicity (Fernando E. Viteri, *et al* 2012). Concern about administering unnecessarily high antenatal iron supplements in women who do not present anemia at the beginning of the second trimester or earlier has been emphasized because of a higher risk of hypertensive disorder and of delivering low birth weight babies, due both to small-for-gestational-age and to premature deliveries (Casanueva E, *et al* 2006), (Ziaei S, *et al* 2007). Importantly, in a report from other study (Ziaei S, *et al* 2007) a high proportion of non-anemic iron-supplemented women with 50 mg daily displayed excessively elevated hemoglobin at term. This condition was associated with elevated rates of preeclampsia and small for gestational age newborns, and other researcher (Lao TT, *et al* 2001) reported a higher incidence of gestational diabetes among non-anemic women receiving antenatal iron compared to those not receiving supplemental iron. Studies done in India and abroad have defined that too much and too less iron doses, both will raise oxidative stress (Neeta Kumar, *et al* 2009).

Table 1: Serum reduced glutathione (GSH) and malondialdehyde (MDA) in preeclamptic pregnant women with and with out supplements .

<i>Parameter</i>	<i>Subject (preeclamptic women)</i>	<i>No.</i>	<i>Mean \pm SD</i>	<i>P-value</i>
GSH(μ M)	Control gp With Vit. C With Folic Acid With Iron	20	156.4 \pm 43	-
		25	168.5 \pm 30	<0.001
		25	161.1 \pm 37	<0.01
		25	154.2 \pm 32	N S
MDA(μ M)	Control gp With Vit. C With Folic Acid With Iron	20	8.89 \pm 1.9	-
		25	7.06 \pm 2.1	<0.005
		25	8.27 \pm 1.8	N S
		25	9.73 \pm 1.7	<0.05

Significant differences when $p < 0.05$, N S : non significant

Table 2: Serum reduced glutathione (GSH) and malondialdehyde (MDA) in preeclamptic pregnant women with Vitamin C supplements .

<i>Parameter</i>	<i>Dose and Period Vit C</i>	<i>No.</i>	<i>Mean ± SD</i>	<i>P-value</i>
GSH(μ M)	≤ 250 mg/day	17	167.3 \pm 37	N S
	> 250 mg/day	8	169.8 \pm 31	
	Early	11	171.6 \pm 28	<0.05
	Late	14	166.5 \pm 32	
MDA(μ M)	≤ 250 mg/day	17	7.52 \pm 2.1	<0.05
	> 250 mg/day	8	6.21 \pm 2.2	
	Early	11	6.42 \pm 1.9	<0.05
	Late	14	7.61 \pm 2.1	

Table 3: Serum reduced glutathione (GSH) and malondialdehyde (MDA) in preeclamptic pregnant women with Folic Acid supplements .

<i>Parameter</i>	<i>Dose and Period Folic Acid</i>	<i>No.</i>	<i>Mean ± SD</i>	<i>P-value</i>
GSH(μ M)	≤ 5 mg/day	18	161.5 \pm 39	N S
	> 5 mg/day	7	162.4 \pm 41	
	Early	21	162.0 \pm 38	N S
	Late	4	161.3 \pm 40	
MDA(μ M)	≤ 5 mg/day	18	8.41 \pm 2.1	N S
	> 5 mg/day	7	7.91 \pm 1.9	
	Early	21	8.11 \pm 1.9	N S
	Late	4	8.65 \pm 1.7	

Table 4: Serum reduced glutathione (GSH) and malondialdehyde (MDA) in preeclamptic pregnant women with Iron supplements .

<i>Parameter</i>	<i>Dose and Period Iron</i>	<i>No.</i>	<i>Mean ± SD</i>	<i>P-value</i>
GSH(μ M)	≤ 200 mg/day	17	155.7 \pm 39	N S
	> 200 mg/day	8	152.1 \pm 41	
	Early	13	151.8 \pm 38	<0.05
	Late	12	156.7 \pm 37	
MDA(μ M)	≤ 200 mg/day	17	8.96 \pm 1.8	<0.05
	> 200 mg/day	8	10.35 \pm 2.2	
	Early	13	10.43 \pm 2.3	<0.05
	Late	12	8.97 \pm 1.9	

Conclusion:

Supplementation with vitamin C especially when using larger doses and early starting in pregnancy can help in improving the health state of preeclamptic women and reducing oxidative stress also using folic acid within pregnancy was very beneficial for decreasing oxidative stress without effected by dose or time of starting and may prevent complication. Regarding the results obtained from study we can conclude that there is no need to use iron supplements for non anemic preeclamptic

women in contrast it will worsen the state and elevate the oxidative stress especially with larger doses and early starting in pregnancy.

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