Relation Between Plasma Levels of Vitamins C and E Antioxidents with Sociodemographic Factors During Pregnancy

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Abstract:

Background: Normal pregnancy induces profound changes in maternal physiology which promotes oxidative stress. Vitamins C and E are antioxidants that work as a defense against this oxidation process.

Objective: To assess the plasma levels of the vitamins C and E at different trimesters of pregnancy and to correlate them with the sociodemographic factors of pregnant women.

Method: A case control study had been conducted in Al-Kadhymia Teaching Hospital which enrolled 120 women. Ninety pregnant cases of 20 -40 years, 30 cases for each of the first, second and third trimesters were the study group and the control group were thirty cases of non-pregnant women of the same age group. Plasma level of vitamin C and E were estimated using high performance liquid chromatography (HPLC)method.

Results: The plasma level of vitamin C was significantly different across the different trimesters of pregnancy and it was significantly lower than the control group P<0.001. Also the plasma level of vitamin C was significantly decreasing with increased parity and age P<0.05, and its level was higher in working pregnant women compared to the pregnant housewives. The plasma level of vitamin E was significantly decreasing through the three trimesters of pregnancy P<0.05.

Conclusion: The plasma level of both vitamins C and E are reduced during pregnancy and life style and occupation have great impact on vitamin C level in plasma. There is significant declining in plasma vitamin C level with parity and increase in maternal age.

Keywords: Vitamins C and E, Antioxidants, Sociodemographic factors, Pregnancy

Introduction:

Oxidative stress refers to excessive generation of reactive oxygen species, which have both physiologic and pathologic roles in the placenta, embryo, and the fetus. Oxidative stress occurs when the generation of reactive oxidant molecules exceeds the capacity of the antioxidants[1,2]. Both enzymatic (Catalase) and non enzymatic (peroxiredoxin, thioredoxin, ascorbic acid or Vitamin C, tocopherol or Vitamin E) antioxidant pathways are present to combat excessive oxidants generation[3,4]. Pregnancy, though a physiological process promot oxidative stress due to the high metabolic activity of placental mitochondria. However, in normal pregnancy there seems a balance between antioxidant and oxidant despite modest oxidative stress[5] . Ascorbic acid and other antioxidants are mediated as defense mechanism against this stress leading to low levels in pregnancy. The degree of reduction in plasma levels has been implicated in aetiology and severity of many obstetric complication[6]. Effects of oxidative stress are seen in pathophysiology of preeclampsia, hydatidiform mole, free radical induced birth defects and other situations such as abortions [7,8] .

Vitamin C also known as ascorbic acid, is a water-soluble vitamin. Unlike animals, humans cannot synthesize vitamin C, rendering its ingestion from exogenous supplement or diet necessary. It is found in a wide variety of fruit) and vegetables. Body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan and contributes to the synthesis of collagen, and the amino acids carnitine and catecholamine that regulate nervous system. It helps to lower blood cholesterol. It is needed for tissue growth and wound healing. [9,10]. Its antioxidant function mainly resides in the protection against lipid peroxidation. Vitamin C as a reducing and antioxidant agent directly reacts with superoxides, hydroxyl radicals, and various lipid hydroperoxides.[3] It's rare to be seriously deficient in vitamin C, although evidence suggests that many people may have low levels of vitamin C. Smokers are at a higher risk of deficiency. A severe form of vitamin C deficiency is known as scurvy. which is characterized by haemorrhages and abnormal bone and dentine formation. Risk factors include Infants who are fed only cow's milk, alcoholism, being elderly and increased need due to increased utilization in pregnant and lactating women [11] Vitamin E is a fat-soluble vitamin with high antioxidant potency. Because it is fat-soluble, α-tocopherol safeguards cell membranes from damage by free radicals. Vitamin C works synergistically with vitamin E to quench free radicals and also regenerates the reduced form of vitamin E[3]. Scientists are investigating whether, by limiting free–radical production, vitamin E might help prevent or delay the chronic diseases associated with free radical .In addition to its activities as an antioxidant, vitamin E is involved in immune function and .as shown primarily by in vitro studies of cells, cell signaling, regulation of gene expression [13] . Frank vitamin E deficiency is rare and overt deficiency symptoms have not been found in healthy people who obtain little vitamin E from their diets [14] premature babies of very low birth weight and

people with fat malabsorption disorders are more likely to become defiant.

The aim of study is to assess the plasma levels of the vitamins C and E at different trimesters of pregnancy and to relate them with the sociodemographic factors of pregnant women.

Patients and methods:

A case control study was conducted at Al-Kadhymia Teaching Hospital in department of obstetric and gynecology; in cooperation with laboratory department during the peroid March 2010 through April 2011.The study was approved by ethical committee of Iraqi scientific council for medical specialization of Obstetrics and Gynecology.

The study included 90 pregnant women selected from antenatal and outpatient clinic of obstetric and gyneacology department. Thirty cases each were recruited from the first, second and third trimester respectively. The control group composed of 30 non-pregnant women of the same age range and parity.

Both the cases and the controls were sampled with questionnaire that documented their ages, parity, occupation, gestational age, drug history, and medical history. The pregnant cases were aged between 20-40 years, and without any medical complications such as diabetes mellitus, hypertension, heart failure or ischemic heart diseases and on no vitamin C and E supplements. The pregnancy confirmed either in the first, second and or third trimester. The gestational age calculated either by last menstrual period or by early ultrasound scan. An informed consent was obtained from all participating women.

Samples collection done by withdrawing of three milliliters of venous blood from each participants. They were separated after centrifugation at 4000 rpm for ten minutes and then the serum stored frozen. After completing samples collection, they were spiked with concentrations ranging from 1000 to 5000 microgram/ml for vitamin C and from 0.25 to 5 microgram/ml for vitamin E .

Laboratory assay: Vitamin C in plasma was extracted as follows: plasma protein was precipitated with 60% methanol and 1 mM EDTA. plasma (100 microliter) was mixed with 400 microliter of 60% methanol/EDTA, incubated for 10 min at 4 degree centigrade before centrifuging at 12,000 rpm for 8 min. The clear phase was transferred to another polypropylene tube and evaporated to dryness under nitrogen. The dried extracts were dissolved in 100 microliter of methanol. Vitamin C and E were separated on the LiChrospher 100 RP-18 column (125x 4mm I.D.; particle size, 5 micrometer), with a mobile phase with a methanol-acetonitriltetra-hydrofuran (75 :20: 5 , v/v/v) at a flow rate of 1.2 ml/min.

Statistical analysis was performed in which the means and standard deviations were determined for quantitative data and frequency determined for categorical variables. Student-t test was used to test for significant association and analysis of variance was used to compare multiple means. ANOVA was used to study the relation between numeric variables. P value < 0.05 was considered statistically significant.

Results:

Table 1 shows the demographic characteristics of study group: among the cases; the working woman were 58, from these the employees were 28 (31.2%) and the farmers were 30 cases (33.3%) while the nonworking (housewives) women were 32 (35.5%). From the employees 3 were doctors, 17 were teachers, and 8 were engineers. Primigravida women (para o) were 38 (42, 2%), para one 27 (30%), para two 9 (10%), para3&4 n=16 (17, 8)

Table 2 shows the mean plasma vitamins C and E in the study and control groups. The mean plasma level of vitamin C in the study group was significantly lower than in control group P=0.001. Also the mean plasma level of vitamin C is significantly different across the three trimesters and it was higher in the 2nd trimester.

The mean plasma level of vitamin E is significantly lower in the study group when compared with control group, P<0.0001. The level of vitamin E was significantly decreasing throughout the three trimesters.

Table 3 shows the relation of plasma levels of vitamins C with occupation, parity and age. The mean plasma level of vitamin C is significantly higher in the working woman (which include the employees and farmers) compared to the non working P<0.05. but it was higher in employees. Also mean plasma level of vitamin C is higher in the young age group compared to older age group P<0.05. and mean plasma level of vitamin C is significantly decreasing with increasing parity, P<0.05

Table 4 shows the Relation of plasma levels of vitamins E with occupation, parity and age. The mean plasma level of vitamin E is significantly higher in the farmers compared to other two groups the employees and housewife P <0.05 the difference between employees and housewife was not significant.

The relation of parity and plasma level of vitamin E showed Positive relationship but not statistically Significant p > 0.05. Also mean plasma level of vitamin E is higher in the young group compared to older age group, had positive non significant relationship, P>0.05.

Table 1: Socio-demographic data of study group

Variables no.of cases percentage
Occupation
Farmer n=30 33.3%
Employee n=28 31.2%
Housewife n=32 35.5%
Parity
P0 n=38 42.2%
P1 n=27 30%
P2 n=9 10%
P3&4 n=16 17.8%
Age group(years)
20-24 n=26 28.8%
25-29 n=22 24.4%
30-34 n=31 34.5%
35-40 n=11 12.3%

Table 2: Mean plasma vitamins C and E in the study and control groups

					P
Vitamin C (mg/ml)	1.73±0.39	1.88±0.38	1.67±0.36	2.27±0.45	0.001
Vitamin E(mg/ml)	1.85±0.5	1.24±0.35	0.95±0.36	2.23±0.43	0.001

All averages are means ±SD

Table 3: Relation of plasma levels of vitamins C with occupation,parity and age

sociodemographic factors mean plasma level of vitamin C mg /ml
Occupation
Employee(n=28) 1.68
Farmers (n=30) 1.567
Housewife (n=32) 1.467
P-value <0.001(S)
Parity of cases
Para 0(n=38) 1.689
Para 1 (n=27) 1.599
Para 2(n=9) 1.505
Para 3&4 (n=16) 1.43
p-value <0.001 [S]
Age group (years)
20-24(n=26) 1.774
25-29 (n=22) 1.699
30-34 (n=31) 1.633
35-40 (n=11) 1.554
p-value <0.001[S]

All averages are mean
NS not significant ; S significant

Table 4; Relation of plasma levels of vitamins E with occupation, parity and age

sociodemographic factors mean plasma level of vitamin E mg/ml
Occupation
Employee(n=28) 0.595
Farmers (n=30) 0.675
Housewife (n=32) 0.59
P-value <0.001[S]

Parity of cases
Para 0(n=38) 1.074
Para 1(n=27) 1.268
Para 2(n=9) 1.415
Para 3&4(n=19) 1.115
p-value >0.06 [NS]
Age group (years)
20-24 (n=26) 1.206
25-29 (n=22) 1.217
30-34 (n=31) 1.225
35-40 (n=11) 1.318
p-value >0.05[NS]

All averages are mean
NS not significant
S significant

Discussion:

It is generally agreed that although pregnancy is a physiological process, it promotes oxidative stress and antioxidants are used as defense mechanism against oxidative stress`[15] . Vitamin E and vitamin C act as antioxidants independent of each other and protect cells according to Madhavi*et al* study which compare it to cells lacking both vitamin C and E[16]

We found in this study that the level of vitamin C in pregnant women group was significantly lower than the control group. In addition the plasma vitamin C level was significantly different across the three trimesters of pregnancy. In this study, the level of vitamin C was higher in the 2nd trimester as compared to the 1st trimester then it decreases again in the 3rd trimester. This may be due to the effects of hemodilution and of active transport of the vitamin to the fetus, which increases throughout pregnancy [14...]The improvement in the nutrition in the2ndtrimester may be the cause for the elevation of the plasma vitamin C level. The vitamin C level decreased again in the 3rd trimester may be due to the increase in fetal and placental demand that enhance the oxidative process and the active transport of the vitamin to the fetus.

These findings are similar to the findings of Uchenna *et al* study and *Rao*eta l **s**tudy who found that serum ascorbic acid was significantly lower in pregnant women than the control but in contrary to our finding they found a progressive fall in concentration of vitamin as pregnancy progressed to third trimester [6,17]

Rev. Bras also found the mean serum concentrations of vitamin C in all the three trimesters were lower than the control but the largest decrease was found in the third trimester, with a 75% drop in the serum total vitamin C concentration (p <0.05) relative to the control. The progressive decline in serum vitamin C concentration observed in this study could indicate an increased utilization of the vitamin by pregnant women to maintain normal reactive oxygen species homeostasis by using the vitamin to mop up the excess reactive oxygen species produced during the pregnancy state[18].

We found significant reduction in the level of vitamin E throughout the three trimester of pregnancy. This is contrary to the findings o**f** *Roes etal* study, who reported a linear increase of vitamin E during pregnancy [19]. The differences in finding may have been due to differences in socioeconomic and nutritional factors of the studied populations. Occupation has great impaction on the social status of the people and their healthcare , hence affecting significantly the plasma levels of ascorbic acid and alpha tocopherol during pregnancy and life in general. This mainly occur because the humans and primates lack L- gulonolactone oxidase from the liver ,rendering them incapable of synthesizing ascorbic acid and thus make them completely dependent on dietary sources of the vitamin [7.]

One of this study findings that the level of vitamin C was significantly higher in the working

women (employees and farmers) compared to the housewives and even it was higher in the employees compared to the farmers This probably because working group have better healthcare (as for employees) and more access to green vegetables and fruits that are rich in vitamin C (as for farmers).On the other hand, vitamin E was significantly higher in the farmers compared to the other two groups(we could not explain why), but the difference in vitamin E level between the employee group and the housewife group was not significant ,probably because vitamin E unlike vitamin C can be stored to a reasonable extent in the liver and other storage depot of the body.

Also during the study, we found that there was inverse relationship between plasma level of vitamin C and the parity, as the level of vitamin C decreases significantly with increased parity. We are unable to explain this relationship but we hypothesized that this may be due to effect of repeated pregnancies on oxidative stress s process and the body in general. In contrast, plasma level of vitamin E show a positive relation with Increased parity but this increase was not significant.

Another finding of this study is the inverse relationship between plasma

level of vitamin C and the maternal age, as the vitamin plasma level decreases significantly with the increase in maternal age. We are unable to explain this relationship but we suggest that it may be due to environmental stress which is likely to be more on older women than on the younger women due to the increase in life responsibilities with increased age. This will require further investigation.

Conclusion:

There is declining in the plasma level of both vitamins C and E during pregnancy despite transient increase in vitamin C level in the 2nd trimester. Lifestyle and occupation have great impact on vitamin C level in plasma. There is significant declining in plasma vitamin C level with parity and increase in maternal age.

Further studies are suggested to evaluate the effect of vitamin C and vitamin E supplementation during uncomplicated pregnancies and also to evaluate their action in management of complicated pregnancies; such as pregnancy associated hypertension and diabetes.

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