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Review Article

Antioxidant Status With Respect To Vitamin E And C In Pregnant Anaemic Women

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ABSTRACT

The present study was conducted to investigate the antioxidant status in iron deficient pregnant anemic women. Forty pregnant women with iron deficiency anemia (IDA) were divided into three groups, namely mild (18), moderate (14) and severe (8) anemic along with pregnant healthy women as controls (20). The levels of haemoglobin, ferritin along with antioxidant Vitamin C and Vitamin E were significantly reduced in all IDA groups. On the basis of our results, it may be concluded that IDA tends to increase the pro oxidant components, which may result in various complications including peroxidation of vital body molecules resulting in increased risk for pregnant women as well as fetus.

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1. Introduction

Iron deficiency anaemia is common anaemia that occurs when iron loss occurs or the dietary intake or absorption of iron is insufficient. In iron deficiency hemoglobin which contains iron cannot be formed. (Brady PG, 2007). It can have deleterious effect on the mother as well as fetus in the form of maternal mortality, intrauterine growth retardation, weight gain, premature labour, preterm delivery, perinatal morbidity and mortality. (Jnnutrition.org content/137/2). Pregnancy is a condition exhibiting increased susceptibility to oxidative stress defined here as a disturbance in the pro-oxidant – antioxidant balance in favour of the former leading to potential damage., (Sies, H. 1991, Page K 1993). Pregnancy mostly because of the mitochondria rich placenta is a condition that favors oxidative stress. Transitional metals especially iron which is particularly abundant in the placenta are important in the production of free radicals (Esther Casanueva 2003). A free radical is a molecule fragment that contains one or more unpaired electron in its outer orbit. Almost all biological macro- molecules are destroyed by free radicals (DM Vasudevan, 2011). Although Oxygen (O₂) is needed for the body, partially reduced form of O₂⁻ (free radicals) and some of their derivatives collectively called reactive oxygen (ROS), are highly toxic pro-oxidant to the body (Singh. K, 1997). It has become increasingly clear that vitamin C and vitamin E play an extraordinary multifaceted role in metabolism of tissues and they help the body ward off many diseases, and protect cell from certain kind of damage, which help them live longer.

Ascorbic acid is a water soluble antioxidant that maintains many metal cofactors in the reduced state. (Rodwell Harpers Biochemistry 2006). Ascorbic acid deficiency affects placental structure and reactive oxygen species and facilitates placental infection, all of which results in increased risk of premature rupture of placental membranes and premature births (Romero, R. Mazar, 1991). It has become increasingly clear that vitamin C and vitamin E play an extraordinary multifaceted role in metabolism of tissues and they help the body ward off many diseases, and protect cell from certain kind of damage, which help them live longer. The placenta is avid to absorb Vit C so that when maternal plasma ascorbic acid concentration is low, it is absorbed by active mechanism. At higher plasma ascorbic acid concentration it enters the placenta by passive diffusion. Vit E is the most important natural antioxidant and appear to be the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipids. (Victor W. Rodwell 2006). Generally a-tocopherol content decrease in the total placenta and in the syncytiotrophoblastic brush border membrane as pregnancy progresses but Vit-E ingestion can elevate it (Yoshioka, T, 1990).

So the present study is planned to evaluate the status of antioxidants in anaemic pregnant patients and to establish the possible use of supplemented ascorbic acid and tocopherol to enhance the absorption of iron supplements and this helps in preventing iron deficiency anaemia in pregnancy which is prevalent in this part of world.

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2. Materials and Method

The study was carried out on 60 subjects ranging in age from 20-40 years out of which 40 were anaemic pregnant women (Iron deficient) & 20 were normal pregnant women.

Distribution Of Subject

Subjects	No of subjects
Normal Pregnant women	20
Iron deficiency anaemia in pregnant women	40

Blood for control & case was collected from the patients coming to MBS hospital Kota and seeking private consultation from the experts engaged in the management of iron deficiency anaemia in pregnancy. Care was taken to ensure that all the control subjects included in study were-

1. Symptomless.
2. Free from any abnormality on routine clinical examination.
3. Free from GIT disorders which effects absorption.
4. Non smoker, Non alcoholic.
5. Not taking any drugs or antioxidants.

These subjects have to undergo-

A. Physical Examination

The physical examination include age, sex, personal clinical history, socio-economic status, literacy, gestational age and finally history of diseases of all subjects included in this study were carefully recorded.

B. Biochemical Examination :

The blood samples were taken by venepuncture from the antecubital vein. Five ml of blood was collected and limited into 2 aliquots. 2.5 ml was transferred to an EDTA coated tube used to determine Hb, MCV and MCH. Remaining 2.5 ml of venous blood centrifuged at 3000 rpm for 15 min. Serum was separated and used for the estimation of vitamin C, E and Ferritin.

Iron assessment can be done by following methods: Hb by Sahalis method. Serum Ferritin by Immunoturbidimetric method. Serum Tocopherol estimation using Bathophenanthroline method. Serum Ascorbic Acid Estimation with Dintirophenyl Hydrazine method.

In the present study 40 patients of iron deficiency anaemia in pregnant women were study. The subjects were those who were attending gynecology & obstetric department MBS, Kota.

Distribution of patients were in 3 groups according to the severity of iron deficiency anaemia.

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In the present study 40 patients of iron deficiency anaemia in pregnant women were study. The subjects were those who were attending gynecology & obstetric department MBS, Kota.

Distribution of patients were in 3 groups according to the severity of iron deficiency anaemia.

The three groups taken are :-

1. Mild
2. Moderate
3. Severe

The following tables and graphs show the different values of serum antioxidant vitamins status i.e. of vitamin C and vitamin E in patients of iron deficiency anaemia in pregnant women.

Table No.-1 Distribution according to age of case & control group subjects

Age group (In Yrs)	Case		Control	
	No.	%	No.	%
<24	25	62.50	13	65.00
>24	15	37.50	7	35.00
Total	40	100	20	100.00

Table No.-2 Distribution according to religion of case & control group subjects

Religion	Case		Control	
	No.	%	No.	%
Hindu	29	72.50	12	60.00
Muslim	11	27.50	8	40.00
Total	40	100	20	100.00

Table No.-3 Mean ± SD of Ht & Wt of case & control group subjects

Parameters	Case	Control	P-value	Significance
Age	24.20±3.67	23.50±2.13	>.05	NS
Ht(Cm)	152.87±2.88	153.10±1.89	>.05	NS
Wt(Kg)	48.85±5.76	52.90±10.49	>.05	NS

Table No.-4 Mean + SD of Vit-C according to religion of case & control group subjects

	Case	Control	P-value	Significance
Hindu	0.44±0.11	0.98±0.25	<.001	HS
Muslim	0.44±0.10	0.99±0.29	<.001	HS

Table No.-5 Mean + SD of Vit-E according to religion of case & control group subjects

	Case	Control	P-value	Significance
Hindu	0.76±0.14	0.99±0.13	<.001	HS
Muslim	0.77±0.14	0.97±0.22	<.001	HS

Table No.-6 Mean + SD of Vit-C according to age group of case & control group subjects

Age group (In Yrs)	Case	Control	P-value	Significance
<24	0.46±0.097	1.07±0.26	<.001	HS
>24	0.41±0.11	0.94±0.21	<.001	HS

Table No.-7 Mean + SD of Vit-E according to age group of case & control group Subject

Age group (In Yrs)	Case	Control	P-value	Significance
<24	0.79±0.13	1.05±0.10	<.001	HS
>24	0.72±0.16	0.93±0.14	<.001	HS

Table No.-8 Mean + SD of Vit-C according to socio economic status of case & control group subjects

SES	Case	Control	P-value	Significance
LIG	0.44±0.10	0.71±0.098	<.001	HS
MIG	0.43±0.11	1.12±0.07	<.001	HS
UIG	0.72±0.16	1.25±0.07	<.001	HS

Table No.-9. Mean + SD of Vit-E according to socio economic status of case & control group subjects

SES	Case	Control	P-value	Significance
LIG	0.73±0.14	0.84±0.04	<.001	HS
MIG	0.76±0.15	1.06±0.08	<.001	HS
UIG	0.72±0.16	1.12±0.02	<.001	HS

Table No.-10. Mean + SD of Vit-C & Vit-E group of case & control group subjects

	Case	Control	P-value	Significance
Vit-C	0.44±0.10	1.03±0.25	<.001	HS
Vit-E	0.76±0.15	1.01±0.13	<.001	HS

Table No.-11 Mean + SD of Vit-C & Vit-E according to severity of case group subjects

	Mean + Sd	
	Vit-C	Vit-E
Mild (n=18)	0.53±0.02	0.88±0.01
Moderate (n=14)	0.43±0.02	0.75±0.06
Severe(n=8)	0.26±0.03	0.50±0.01

Vit-C	Vit-E
Control v/s Mild P>.05 NS	Control v/s Mild P>.05 NS
Mild v/s Moderate P>.05 NS	Mild v/s Moderate P>.05 NS
Mild v/s Severe P<.001 HS	Mild v/s Severe P<.001 HS
Moderate v/s Severe P>.05 NS	Moderate v/s Severe P<.001 HS
Control v/s Severe P<.001 HS	Control v/s Severe P<.001 HS
Control v/s Moderate P<.001 HS	Control v/s Moderate P<.001 HS

Table No.-12. Correlation between Vit-C and Vit-E of control group subject

Correlation	r-value	p-value	Significance
Vit-C v/s Vit-E	+0.856	<.001	HS

Table No.-13 Correlation between Vit-C and Vit-E of case group subject

Correlation	r-value	p-value	Significance
Vit-C v/s Vit-E	+0.954	<.001	HS

In the present study we observed that the mean level of vitamin C in anaemic pregnant subject was (0.44 ± 0.10) lower than the normal subjects (1.03 ± 0.25) it shows that the mean level of vitamin C significantly decreased in the anaemic subject as compared to the normal subjects. Hence, the difference between the two sample's mean statistically differs significantly i.e. $P < 0.001$ (Table No. 10). The study further reveals that the mean level of vitamin C was lower in anaemic pregnant age <24 that is (0.46 ± 0.097) than normal subjects that is (1.07 ± 0.26).

The mean level of vitamin C was lower in anaemic pregnant subjects of age >24 i.e. (0.41 ± 0.11) than normal subjects i.e. (0.94 ± 0.21) so the difference between the two samples mean statistically differ significantly i.e. $P < 0.001$ (Table No. 6). The mean level of vitamin C in anaemic pregnant subject in LIG, MIG, UIG was (0.44 ± 0.10), (0.43 ± 0.11) and (0.72 ± 0.16) respectively which was lower than the normal subjects in LIG, MIG, UIG i.e. (0.71 ± 0.098), (1.12 ± 0.07) and (1.25 ± 0.07) respectively. The mean level of vitamin C in mild anaemia i.e. (0.53 ± 0.02) was higher as compared to the mean level of moderate and severe anaemia i.e. (0.43 ± 0.02) and (0.26 ± 0.03) respectively.

The difference between mean level of vitamin C in control v/s mild ($p > .05$), mild v/s moderate ($p > .05$) and Severe v/s moderate ($p > .05$) were non significant. Mild v/s severe and control v/s severe i.e. $p < .001$, control v/s moderate were highly significant. (Table No. 11).

It was observed that as the severity of anaemic increase, the mean level of vitamin C decreases. The comparative levels of plasma ascorbic obtained in anaemic pregnant subjects and controls in the present study were a true reflection of the serum level of vitamin, the lower levels in the anaemic pregnant would represent a relative iron deficient state which may result in the prolongation of the symptoms. If the results obtained in the present study are confirmed in larger series and elsewhere, there may be case for routine administration of ascorbic acid to anaemic pregnant subjects in an effort to reduce the severity of anaemia. Attention needs to be given to measure both tissue and plasma vitamin C because the two are not necessarily related and because oxidant stress can affect tissue concentrations.

It is only through a knowledge of tissue concentrations that the efficacy of supplementation can be reliably ascertained and Vitamin E is highly effective fat-soluble vitamin with a variety of cellular membrane stabilizing antioxidant and non-antioxidant functions. Vitamin E has been suggested to prevent the oxidation of polyunsaturated fatty acids in red blood cell (RBC) membrane, thus inhibiting the premature erythrocytolysis. Animal studies have shown that treatment with vitamin E results in increase number of colony forming units of erythroid precursors, enhanced erythropoiesis and improved blood hemoglobin levels in these animals. Several clinical trials have indicated that vitamin E might be used therapeutically as a potential erythropoietic agent for decreasing the premature erythrocyte hemolysis by reducing the fragility of erythrocytes.

By this way, it improves the post-supplemental blood hemoglobin and hematocrit levels in some of the anaemic human subjects, including low birth weight premature infants, patients suffering from various inherited hemolytic anemia, chronic renal failure patients on hemodialysis and apparently healthy mildly anaemic subjects. (Tanveer Jilani, Mohammed Perwaiz Iqbal 2011). In present study we observed that the mean level of vit. E in anaemic pregnant subjects was (0.76 ± 0.15) which was lower than the normal subjects (1.01 ± 0.13). It shows that the mean level of vitamin E significantly decreased in the anaemic pregnant subjects as compared to the normal subjects hence, the difference between the two samples mean statistically differ significantly i.e. $p < .001$ (table no 10). The study further reveals that the mean level of vitamin E was lower in anaemic pregnant subjects of age <24 years i.e. (0.79 ± 0.13) than normal subjects that is (1.05 ± 0.10) and in age group >24 years mean level of vitamin E was lower in anaemic pregnant subjects i.e. (0.72 ± 0.16) than normal subjects i.e. (0.93 ± 0.14).

Hence the difference between the two samples mean statistically differ significantly $p < .001$ (table no 7). The mean level of vitamin in anaemic pregnant women of LIG was lower (0.73 ± 0.14) than normal subjects (0.84 ± 0.04). The level of vitamin E in anaemic pregnant subjects in MIG and UIG was low i.e. (0.76 ± 0.15) and (0.72 ± 0.16) respectively than normal subjects i.e. (1.06 ± 0.08) and (1.12 ± 0.02) respectively. Hence the difference between the two samples mean statistically differ significantly $p < 0.001$ (table no 9). The mean level of vitamin E (0.88 ± 0.01) in those with mild anaemic subjects was higher as compared to the mean level of those with moderate and severe anaemia i.e. (0.75 ± 0.06) and (0.50 ± 0.01) respectively (Table No 11). The difference between mean level of vitamin E in mild v/s moderate, mild v/s severe, and moderate v/s severe, control v/s severe and control v/s moderate were highly significant i.e. $P < 0.001$ (table no 11). The mean level of vitamin E in control v/s mild i.e. ($p > .05$) and mild v/s moderate was non significant. The study was planned to estimate the antioxidant vitamin C and vitamin E levels in IDA patients and to ascertain whether their supplementation helps to improve this condition.

From the results of this study following conclusion were drawn.

1.. The aim of the present study was to find out the serum antioxidant status of vitamin C and Vitamin E in pregnant anaemic patients and to observe that if there is any correlation between serum ascorbic acid and serum tocopherol level in pregnant anaemic patients according to severity.

2.. 60 subject ranging from 20-40 years in age were chosen out of which 20 were normal pregnant and 40 were anaemic pregnant subjects.

3.. Subjects chosen were those who were attending the ANC lab, MBS hospital, Kota. Subjects were excluded from the study who were taking antioxidant medications already.

4. Serum ascorbic and serum tocopherol status along with routine tests were estimated.

5. A comparison of mean value was made

(a) Between level of serum ascorbic acid of normal and anaemic pregnant subject according to age.

(b) Between level of serum tocopherol of normal pregnant and anaemic pregnant subjects according to age.

(c) Between level of serum ascorbic acid & severity of anaemia in pregnant subjects.

(d) Between level of serum tocopherol and severity of anaemia in pregnant subject.

6. Statistical analysis was done by students t' test.

7. From the observation we concluded that –

(a) Serum ascorbic acid was significantly decreased in anaemic pregnant subject as compared to normal subject (i.e. $p < 0.001$). The decrease in endogenous ascorbic acid may be due to its extensive use as antioxidants to protect the gastrointestinal tract from the free radical damage during oxidative stress. Hence supplementation of vitamin C is required in iron deficiency anaemia.

(b) Serum tocopherol level were significantly decreased in anaemic pregnant subject as compared to the normal subjects (i.e. $p < 0.001$). Vitamin E is a chain breaking antioxidant involved in the inhibition of propagation of free radicals generation during IDA. So present study indicates that vitamin E requirements is increased in IDA disorder and supplementation of vitamin E may help in the treatment of the diseases.

© In case of mild anaemia level of serum ascorbic acid was found higher than those of moderate & severe anaemia. The difference between the of mean level of serum ascorbic level in control v/s severe, mild v/s severe, moderate v/s control were highly significant.

(d) The difference between the mean level of serum tocopherol in control v/s severe, mild v/s severe, and moderate v/s severe and control v/s moderate anaemic pregnant patients were highly significant i.e. $0 < .001$.

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