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COMPARISON OF SERUM ANTIOXIDANT (ASCORBIC ACID AND TOCOPHEROL) LEVELS IN ANTENATAL WOMEN WITH AND WITHOUT PRETERM PREMATURE RUPTURE OF MEMBRANES .

Obstetrics And Gynecology

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ABSTRACT

Background :Preterm premature rupture of membrane (PPROM) is a global contributor to preterm births and associated maternal morbidity and perinatal morbidity and mortality . The exact cause of PPROM is unknown .Several studies point towards Reactive oxygen species (ROS) as major contributor and in vitro studies have proved that antioxidants like Ascorbic acid and Tocopherol have synergistic protective effect against ROS induced damage.
This study hypothesized that PPROM occurs due to decline in these antioxidant levels.
Method :97 antenatal women with PPROM were selected from 5672 antenatal women admitted during a period of one year at our tertiary care teaching hospital . S. Ascorbic acid was estimated in 60 cases and 100 controls while S . Tocopherol levels in 55 cases (60 controls) by method of Omaye et al and modified method of Omu et al. respectively.
Results: Incidence of PPROM in the study was 1.7 % . The mean S. Ascorbic acid in cases was 0.726 mg/dl which was significantly lower than the mean value of controls (1.434 mg/dl). The mean value of S. Tocopherol in cases was 0.248 mg/dl which was also significantly lower than the mean value of 0.515 mg/dl among controls. It was observed that S. Ascorbic acid levels were significantly higher among women with longer leaking-delivery interval.
Conclusion :Serum levels of both ascorbic acid and tocopherol were found to be significantly lower in pregnant women with preterm premature rupture of membranes.

KEYWORDS

Preterm Labor, Vitamin C, Vitamin E, Rupture Of Amniotic Membranes

INTRODUCTION:

Preterm premature rupture of membranes (PPROM) denotes the spontaneous rupture of fetal membranes that occurs before 37 completed weeks of gestation and before onset of labour. PPROM accounts for 1-2% of all births and is associated with 20-30 % of all preterm deliveries and 20 % of all perinatal mortality. Effective means to prevent preterm birth reasonably depends on identification and remediation of pathophysiological factors that cause PPROM.

The exact cause of PPROM is not yet known. Several lines of investigation point towards damage due to Reactive oxygen species (ROS) .It is likely that PPROM has variety of causes but many believe intrauterine infections to be one of the major predisposing events (Gomez and collegues , 1997[1];Mercer et al , 1997[2]). In a review of 10 studies involving 800 patients with PPROM who underwent amniocentesis for bacterial culture analysis,32.1 % resulted in positive cultures. McGregor et al[3] demonstrated in vitro that multiple metalloproteinase producing bacteria including Group B streptococcus and prevotella species, can impair measures of membrane strength and elasticity predisposing to PPROM . Smoking is considered to be a leading risk factor for PPROM. Tobacco smoke contains a complex mixture of smoke-borne ROS and aromatic hydrocarbons that induce systemic oxidative damage to multiple tissues .Smoking causes depletion of antioxidants from amnion and chorion leaving them vulnerable to ROS induced damage . The oxidative stress caused by increased ROS formation and /or antioxidant depletion may disrupt collagen and cause premature rupture of membranes by causing focal collagen damage in fetal membranes.

ROS are continuously generated through leakage from electron transport chain of cellular respiration within the mitochondria and are major factors associated with tissue damage. Fetal membranes derive their strength from collagen. The ROS disrupt the helix cross-bridging of collagen besides inhibiting tissue inhibitors of metalloprotienases (TIMPS) and weaken the collagen structure as well as increase damage due to metalloprotienases. Antioxidants counteract this effect. There are a number of antioxidants available but ascorbic acid and tocopherol are mainly responsible for membrane stability. Plessinger et al(4) developed an in vitro exposure system with term human amnion chorion membranes obtained from placentae of 4 women with elective repeat caesarean section without labour. Sections of amnion and chorion were exposed to hypochlorous acid after incubation with phosphate buffer and saline and some after incubation with ascorbic acid and Tocopherol (trolox C) . Hypochlorous acid caused dose dependent destruction to the amniotic epithelium and collagen I. This was prevented by pre-incubation with Ascorbic acid and Tocopherol. This in vitro finding indicates that Ascorbic acid and Tocopherol have preventive effect against ROS induced damage to amniotic membranes.

It is hypothesized that PPROM occurs due to decline in antioxidant levels but review of literature shows minimal evidence with human studies proving the same. This study was therefore conducted to estimate the levels of serum Ascorbic acid(Vitamin C) and Tocopherol (Vitamin E) in patients with PPROM and compare it with gestational age matched antenatal women without PPROM .

MATERIAL AND METHOD:

The study was conducted in the Obstetrics and Gynecology Department in collaboration with Biochemistry Department of King George's Medical University, Lucknow over a period of 1 year. The cases and controls were both selected from the women attending the antenatal clinic of Obstetrics and Gynaecology Department.

During the study period, 97 women presented with history of watery discharge per vaginum between 20 weeks to 36 weeks and 6 days without onset of labor. PPROM was confirmed by clinical examination (abdominal and per speculum). Women were screened for exclusion criteria (chronic debilitating disease, immunocompromised state, severe anaemia, congenitally malformed fetus, polyhydramnios, multiple pregnancy, cervical incompetence, intrauterine growth retardation, history of trauma). Ultrasound examination was done to confirm amount of liquor and rule out congenital anomaly. Urogenital infections were detected through urine and vaginal culture sensitivity tests.

100 antenatal women without PPROM and not in labor who were matched by age and gestational age and were recruited as control group.

Out of 97 women, 77 were included in the study group. 10 cases were excluded due to twin pregnancies (4), polyhydramnios (1), severe anaemia (2), preterm labour prior to rupture of membranes (3) and 10 cases were lost to follow up.

Informed and written consent was taken from all participants and ethical clearance was obtained from institutional ethical committee.

Ascorbic acid and Tocopherol level estimation -
3ml of venous blood were collected in plain vials with aseptic precautions. Serum was separated and estimated for levels of Ascorbic acid and Tocopherol at the Biochemistry Department.

Serum ascorbic acid was estimated in 65 cases and 100 controls by spectrometry as per method by Omaye et al. (1979) in which equal parts of homogenate and water were centrifuged with tricarboxylic and then incubated with reagent at 37 degree centigrade for 3 hours . Then it was mixed with sulphuric acid and the color developed read at 520mm Shimadzu ultraviolet spectrometer.

Serum Tocopherol levels was measured in 55 cases and 60 controls by deriving hexane extract after mixing serum with hexane and centrifuging for 5 minutes .This preparation was then injected into HPLC (High pressure liquid column) as per modified method of Omu et al. (1999).

Data Management and Statistical Analysis
Data was presented as range, mean and percentages and were analysed using SPSS-10 software.

RESULTS

The total number of pregnant women admitted during the study period was 5672. Out of these, 97 were admitted for PPROM. Thus, the incidence of PPROM was 1.7%.

Table 1 shows demographic and gestational age distribution of cases and controls. Women belonged to the age range 20 to 35 years and the majority of them were between 20 to 25 years both in cases (67%) and controls (65%). Parity-wise the maximum subjects were primigravida(P0) both in cases (68%) and controls (75%) .

77.3% cases and 68% controls were in the gestational age group 31 to 36weeks 6 days .56.7% of cases and 57% of controls belonged to low socio-economic status.

Low socio-economic status (56.7%) and infection (58.7%) were the most prevalent risk factors among cases.

Table 2, shows the comparison of mean serum Ascorbic acid and serum Tocopherol levels between cases and controls .The mean values were significantly lower ($p<0.01$) for both Ascorbic acid (0.726 mg/dl) and Tocopherolol (0.515 mg/dl) in cases as compared to controls.

Pregnant women with prolonged leaking-delivery intervals had higher mean serum Ascorbic acid levels (**Table 3**). This difference was found to be statistically significant.($p=0.035$) for Ascorbic acid.

It was observed that mean Ascorbic acid value in cases of PPROM of duration more than 24 hours was significantly higher as compared to those with onset of labour within 12 hours ($p=0.027$). A significant difference ($p=0.048$) in mean ascorbic acid levels was seen between those with onset of labour within 12-24 hours and beyond 48 hours . No correlation found between serum tocopherol levels and leaking delivery interval.

As shown in **Table 4**, S. Ascorbic acid levels were significantly lower in cases as compared to controls across all age groups , among primigravidas and in the gestational age between 26 to 36 weeks 6 days . Tocopherol levels were significantly lower in cases of all age and gestational age groups and among primigravidas. S. Tocopherol levels were also significantly lower in cases of all gestational age groups and among primigravidas.

DISCUSSION

The incidence of PPROM in our study (1.7%) is similar to the incidence reported in various studies (1-2%) (5) .The condition is more common in first pregnancy and younger age group. Anna Maria Siega et al (5) found that 65 % of their cases were aged between 20-29 years and 48% were primigravidae. In the present study too, 67% of cases were aged between 19 -25 years and 68% were primigravidae. Apart from these two factors the other demographic factors have no association with incidence of PPROM . **Table 1.**

Barett et al. (6) estimated amniotic fluid levels of Ascorbic acid in 80 pregnant women and found that the levels to be significantly lower in women with PPROM as compared to controls ($p=0.025$) . Wideman et al (7) showed that low maternal blood and leucocyte levels of Ascorbic acid are associated with increased risk of PPROM . In a prospective study by Casaneuva et al. (8), leucocyte ascorbic acid levels were measured in pregnant women at 20, 28 & 32 weeks gestation. Low Ascorbic acid levels at 20 weeks gestation identified women with an increased incidence of PPROM ($p<0.05$). In a prospective study done by Richa Sharma et al. (9), Ascorbic acid levels were low in women with PPROM (0.41 ± 0.08 versus 0.84 ± 0.19mg/dl) as compared to controls .In the present study, the mean serum ascorbic acid levels were found to be significantly lower in cases of PPROM as compared to controls ($p <0.001$) supporting the previous studies.This significant difference was found in different subgroups of age and parity for both Ascorbic acid and Tocopherol levels .(Table 4)

Serum Tocopherol levels were studied by Barett et al. (10) in serum and amniotic fluid and no significant difference was found in women with and without PPROM. The present study however shows that mean serum Tocopherol levels are significantly lower ($p<0.001$) in women with PPROM (0.248 mg/dl) as compared to women without PPROM (0.515 mg/dl). There is a definite paucity of research on serum tocopherol levels. Tocopherol has mostly been considered in conjunction with Ascorbic acid.

Richa Sharma et al (9) observed inverse relationship between duration of rupture of membranes and vitamin C levels. The present study however showed that the vitamin C levels were higher in women with longer duration of leaking and they had longer latency period. This difference was found to be significant ($p=0.027$). The latency period had no such significant correlation with serum tocopherol levels . (Table 3).

Gestational age effects the antioxidant levels .Maternal serum Ascorbic acid levels decline normally in pregnancy until third trimester. Casaneuva et al. attributed this decline to the effect of hemodilution during pregnancy. Garba et al (11) estimated serum Ascorbic acid concentration in 90 pregnant women and observed it to be 2.55 ± 0.82 mg/dl, 2.32 ± 0.40 mg/dl and 0.27 ± 0.1 mg/dl in first, second and third trimester respectively. Relative to serum Ascorbic acid in controls (3.15 ± 0.13 mg/dl), these values were significantly lower ($p<0.05$). Though ,in present study mean levels of serum ascorbic acid and tocopherol levels were significantly lower in cases in all gestational age groups compared to controls , no statistically significant pattern of decline in the levels were found among cases and controls when gestational age groups were compared .

Richa Sharma et al (9) found there is a linear decline in plasma vitamin C levels as the pregnancy advances. This study did not find any such linear pattern of decline in either Vitamin C or E levels.

In a prospective cohort study conducted by Anna Maria Siega et al (5) vitamin C intake preconceptionally and during second trimester was examined in 2064 women with singleton pregnancies for its association with PPROM and preterm labour.It was found that women who had total vitamin C intakes $<10^{th}$ percentile preconceptionally had twice the risk of preterm delivery because of preterm rupture of membranes .Some studies on supplementation show an indirect evidence of low levels of these antioxidants in women with PPROM .In a randomized trial , Casaneuva et al (12) recruited 120 women with singleton pregnancy after 20 weeks of gestation .68 women were randomized to receive placebo and 58 received 100 mg of ascorbic acid daily. Incidence of PPROM was 8% in ascorbic acid group and 25% in placebo group. Nayereh Ghomian et al (13) studied the role of

vitamin C in prevention of preterm premature rupture of membranes and concluded vitamin C supplementation daily from 14th week of gestation significantly decreased the incidence of PPROM in cases as compared to the group.

All these findings are consistent with the biological action of ascorbic acid to stimulate synthesis and strengthen collagen , while protecting against damage by ROS. Tocopherolol seems to have the same protective effect as suggested by lower levels of in cases of PPROM as compared to controls.

Our study supports and confirms that there is deficiency of Ascorbic acid and Tocopherol levels in PPROM cases which may be the cause of PPROM . Hence, supplementation could be recommended in pregnancy in those women with deficiency of vitamin C during pregnancy ,as it is a water-soluble safe vitamin, safe in pregnancy without risk of hypervitaminosis . Yet other known risk factors for PPROM like genitourinary tract infections should be promptly treated .

The safety of vitamin E in pregnancy is not clearly known and it needs more evidence for recommendation for its supplementation in pregnancy.

CONCLUSION:

Serum levels of both Ascorbic acid and Tocopherol are significantly lower in cases of PPROM. Low ascorbic acid levels are also associated with shorter latency period for delivery.Thus , Vitamin C supplementation should be advocated to all antenatal women for prevention of PPROM.

Table 1: Demographic And Gestational Age Distribution Of Cases And Controls

	Study group n=90		Control group n=100		p value
Age (yrs)	**Number**	**%**	**Number**	**%**	
19—25	66	68.04	75	75	0.279
26—30	16	16.49	16	16	0.925
31—35	15	16.46	9	9	0.165
Parity					
P_0	65	67.01	65	65	0.766
P_1	16	16.49	15	15	0.773
$\geq P_2$	16	16.49	20	20	0.706
Religion					
Hindu	88	90.72	88	88	0.536
Muslim	9	9.28	12	12	
Socioeconomic Status					
Low	55	56.7	57	57	0.966
Middle	42	43.3	40	40	0.639
High	—	—	3	3	0.085
Gestational Age					
20 —25 Wks 6 days	8	8.25	10	10	0.66
26 — 30 wks 6 days	14	14.43	22	22	0.169
31 — 36 wks 6 days	75	77.32	68	68	0.143

Table 2 .mean Serum Ascorbic Acid And Tocopherol Levels

	S. ASCORBIC ACID LEVELS (mg/dl) n=65		S. TOCOPHEROL LEVELS (mg/dl) n=55	
	CASES	CONTROL	CASES	CONTROL
RANGE	0.01 — 1.36	0.76 — 2.90	0.06 — 0.84	0.1 — 1.62
MEAN	0.726 ± 0.33	1.434 ± 0.557	0.248 ± 0.177	0.515 ± 0.290
p value	< 0.001		3.46 x 10-8	

Table 3: Correlation Of Mean Serum Ascorbic Acid And Serum Tocopherol Levels With Pprom-delivery Intervals (latency Period)

LATENCY PERIOD	S. ASCORBIC ACID LEVELS (mg/dl) n=65	S. TOCOPHEROL LEVELS (mg/dl) n=55
<36 hours	0.577 ± 0.409	0.207 ± 1.66
36 hours to 7 days	0.736 ± 0.301	0.289 ± 0.201
>7 days	0.856 ± 0.254	0.205 ± 0.108
p value	0.04	0.23

Table 4. Correlation of S.Ascorbic acid and S. Tocopherol levels in cases and controls with age, parity and gestational age.

Age (years)	**Mean** ascorbic **acid levels**			**Mean Tocopherol levels**		
	Study group (n=65) Mean=0.726	Control group (n=100) Mean=1.434	"p" value	Study group (n=55) Mean=0.248	Control group (n=60) Mean=0.515	"p" valve
19-25	0.699	1.428	**<0.001**	0.269	0.525	**<0.001**
26-30	0.746	1.369	**0.011**	0.205	0.426	**0.009**
31-35	0.866	1.649	**0.003**	0.216	0.492	**0.007**
Parity						
P_0	0.672	1.462	$\mathbf{3.01x10^{-13}}$	0.254	0.519	**<.0001**
P_1	0.837	1.372	0.002	0.289	0.519	0.140
$\geq P_2$	0.799	1.394	0.009	0.186	0.502	0.001
Gestational Age						
20-25 wks 6 days	0.998	1.813	0.0003	0.254	0.740	**0.017**
26-30 wks 6 days	0.614	1.923	$\mathbf{6.39x10^{-5}}$	0.291	0.301	0.918
31-36 wks 6 days	0.684	1.236	$\mathbf{7.79x10^{-12}}$	0.243	0.503	**<0.001**

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