

# Total Free Radical Trapping Antioxidant Potential in Normal Pregnancy

Kharb Simmi<sup>1</sup>, Singh Veena<sup>2</sup>

<sup>1</sup>Lecturer, <sup>2</sup>Associate Professor, Department of Biochemistry, Pt. B.D. Sharma PGIMS, Rohtak, Haryana

**OBJECTIVES** – To find out the total radical trapping capacity (TRAP) of serum antioxidants in normal pregnancy. **METHODS** – Serum tocopherol (VE), ascorbate (VC), urate (SU), malonaldehyde (MDA) and total thiol (SH) levels were estimated and analysed in 25 normotensive healthy pregnant women and in 25 non-pregnant healthy volunteers acting as controls. **RESULTS** – Serum vitamin C (VC), and E (VE) and total thiol (SH) levels were lower in normal pregnant women as compared to those in controls ( $p < 0.001$ ). MDA was significantly increased in pregnant women ( $p < 0.05$ ). Also, TRAP calculated was higher in pregnant women but non-significantly so. **CONCLUSION** – TRAP test provides a means of assessing the total capacity of the chain-breaking antioxidants to prevent lipid peroxidation in plasma.

**Key words** : free radicals, antioxidants, TRAP, pregnancy, vitamin C, vitamin E, uric acid, malonaldehyde, thiol

## Introduction

Low levels of lipid peroxidation are essential to many cellular processes since small amounts of lipid peroxides and their semistable break-down products act as intracellular and extracellular messengers<sup>1</sup>. Although a variety of antioxidant mechanisms serve to control lipid peroxidation, under certain conditions the protective mechanisms can be overwhelmed leading to elevated steady-state tissue levels of peroxidation products<sup>1</sup>. The total antioxidant capacity in man is dependant on synergistic action of various antioxidants<sup>2</sup>. A test has been developed to measure the total radical trapping capacity in plasma<sup>3</sup>. It has been reported that in normal pregnancy, there is an increase of lipid peroxidation products in serum as gestation progresses<sup>4</sup> and this finding correlates with changes in lipoperoxidation activity of placenta<sup>5</sup> and with an increase of total serum lipids<sup>6</sup>. Scanty and controversial data are available on the antioxidant capacity in pregnancy. It has been reported that lipid peroxidation is controlled by an adequate antioxidative response during normal pregnancy<sup>7</sup>. Hence, the present study was undertaken to measure the serum antioxidants - urate, tocopherol, and ascorbate, and to calculate total radical trapping capacity (TRAP) in normal pregnancy.

## Material and Methods

Twenty-five normotensive healthy pregnant women (19-35 years old, in 32-40 weeks gestation) attending the Outpatient Department of Obstetrics and Gynecology were selected for the study and 25 age matched, non-

pregnant healthy volunteers served as controls. Venous blood was drawn and serum was separated by centrifugation. Uric acid (UA) was estimated colorimetrically<sup>8</sup>, vitamin C (VC)<sup>9</sup>, vitamin E (VE) and malonaldehyde (MDA) levels were analysed<sup>10</sup>. Total plasma sulphydryl concentration (SH) was measured by Ellman's reaction<sup>11</sup>. Thus, from the measured concentration of the serum antioxidants TRAP was calculated<sup>12</sup>.

$TRAP (\mu\text{mol/L}) = 1.3 [UA] + 1.7 [VC] + 2.0 [VE] + 0.2 [SH]$ .

The data so obtained was analysed statistically using student's 't' test.

## Results

Serum vitamin C and E and total thiol (sulphydryl) concentrations were lower in normal pregnant women as compared to those in non-pregnant controls ( $P < 0.01$ , Table I). MDA, product of lipid peroxidation, was significantly increased in the pregnant group as compared to that in the control group ( $p < 0.05$ ). TRAP calculated was higher in pregnant women as compared to that in controls but not significantly so.

**Table I: Parameters of oxidative stress (mmol/L, mean  $\pm$  SD)**

	Non-pregnant (n=25)	Normal pregnancy (n=25)	p-value
Uric acid	175.23 $\pm$ 32.45	295 $\pm$ 14.75	<0.01
Vitamin C	113.56 $\pm$ 10.22	60.18 $\pm$ 23.27	<0.01
Vitamin E	25.75 $\pm$ 0.89	23.63 $\pm$ 0.97	<0.01
Thiols	449 $\pm$ 37	393 $\pm$ 112	<0.01
TRAP	562.14 $\pm$ 228	611.67 $\pm$ 83.08	NS
MDA	1.14 $\pm$ 0.07	1.78 $\pm$ 0.22	<0.05

Paper received on 27/11/02 ; accepted on 06/04/04

Correspondence :

Dr. Simmi Kharb

11, No. 1447, Sector - 1, Urban Estate,

Rohtak - 124001, Haryana.

Email: simmikh@rediffmail.com

## Discussion

We observed statistically insignificant higher TRAP values in pregnant women as compared to those in controls and this can be because of higher serum urate concentrations in the pregnant group. Differences in urate concentrations in normal pregnant women and those in non-pregnant ones have been documented previously<sup>13</sup>. In women with congenital xanthine dehydrogenase/oxidase deficiency in whom uric acid was usually undetectable in the blood, plasma uric acid increased during pregnancy<sup>14</sup>. Urate is the major contributor to TRAP, accounting for between 38-47% of the total<sup>12</sup>. In contrast, antioxidant vitamin C and E contribute 13 to 17% and 2 to 8% respectively. We found low vitamin C and E levels in pregnant women as compared to those in controls which is in keeping with other studies. Ascorbic acid has been described as the major "frontline" water-soluble antioxidant. Vitamin C can regenerate vitamin E from the alpha tocopheroxyl radical; however, this is not the case for urate and glutathione<sup>12</sup>. Urate possesses preventive antioxidant activity through metal ion chelation in addition to chain breaking activity.

The sulfhydryl groups have the highest plasma concentration of all antioxidants, but act as relatively ineffective antioxidants with very low percentage contribution to TRAP. No statistically significant difference in thiol levels was observed between pregnant and non-pregnant groups, though the values were slightly lower in the pregnant group. The oxidative destruction of essential sulfhydryl groups of proteins and enzymes in the presence of lipid hydroperoxides has been reported<sup>3</sup>. Findings of low thiol and high MDA levels in pregnant women in the present study could be because of this fact.

Free radical chain oxidation and the interaction of various antioxidants are now attracting the attention of nutritionists. The important role of vitamin C and E and uric acid in TRAP during pregnancy suggests that changes in their concentration may influence susceptibility of vascular endothelium to oxygen toxicity.

Thus, TRAP test may provide a means of assessing the total capacity of the chain-breaking antioxidants to prevent lipid peroxidation in plasma.

## References

1. Cheeseman KH, Slater TF. An introduction to free radical biochemistry. *Br Med Bull* 1993;49:481-93.
2. Wilson RL. Free radical production: why vitamin E, not vitamin C, B carotene or glutathione? *In: Biology of Vitamin E. Ciba Foundation Symposium 101, Pitman Books, London 1983; 19-44.*
3. Wayner DDM, Burton GW, Ingold KU et al. The relative contributions of vitamin E, urate, ascorbate and proteins to the total radical trapping antioxidant activity of human blood plasma. *Biochim Biophys Acta* 1987;924:408-19.
4. Maseki M, Nishigaki I, Hagihara M et al. Lipid peroxide levels and lipid serum content of serum lipoprotein fractions of pregnant subjects with or without preeclampsia. *Clin Chim Acta* 1981;115:155-61.
5. Diamant S, Kissilevitz R, Diamant Y. Lipid peroxidation in human placental tissues. General properties and influence of gestational age. *Biol Reprod* 1980;23:776-81.
6. Hubel CA, Roberts JM, Taylor RN et al. Lipid peroxidation in pregnancy : new perspectives on preeclampsia. *Am J Chim Acta* 1989; 161: 1025-34.
7. Uotila JT, Tuimala RJ, Aarnio TM. Findings on lipid peroxidation and antioxidant function in hypertensive complications of pregnancy. *Br J Obstet Gynecol* 1993; 100:270-6.
8. Henry RJ, Sobel C, Kin J. Estimation of uric acid in blood. *Am J Clin Path* 1957; 28:153-7.
9. Roe HJ. Ascorbic acid in blood and urine. *Standard Methods of Clinical Chemistry* 1961;3:35-45.
10. Kharb S, Gulati N, Singh V et al. Lipid peroxidation and vitamin E levels in preeclampsia. *Gynecol Obstet Invest* 1998;46:238-40.
11. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
12. Lindeman HJ, Grobden DVZ, Schrijver J et al. The total free radical trapping ability of cord blood plasma in preterm and term babies. *Pediatr Res* 1989;26:20-4.
13. Many A, Hubel LA, Robberts JM. Hyperurecemia and xanthine oxidase in preeclampsia, revisited. *Am J Obstet Gynecol* 1996;174:288-91.
14. Beaufile M, Uzan S, Don Simoni R et al. Metabolism of uric acid in normal and pathologic pregnancy. *Contrib Nephrol* 1981; 25:132-6.