Some Biochemical Markers of Oxidative Stress in Pregnant Nigerian Women.

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Abstract

Context: The increased generation of free radicals and reactive oxygen species in pregnancy results in oxidative stress. Antioxidants are used as defence mechanisms and their serum concentrations give oxidative stress levels.
Objective: To determine serum levels of ascorbic acid, uric acid and bilirubin (antioxidants) , as simple markers of oxidative stress in pregnant Nigerian women.
Study Design, Setting, Subjects and Methods: Pregnant women numbering sixty-five, aged between 20-38 years, in their second and third trimesters were selected from the antenatal clinic, University of Nigeria Teaching Hospital (UNTH) Enugu. A comparable group of 65 non-pregnant women (controls) were also recruited. Serum levels of ascorbic acid were determined by Roe and Kenther method, uric acid by Brown's method and bilirubin by Powell's method.
Results: The serum levels of ascorbic acid, uric acid and bilirubin were each significantly lower in pregnant than in non-pregnant control subjects ($p < 0.05$ in each case). There was also a steady decline in the serum levels of the antioxidants with increasing gestational age.
Conclusion : Antioxidant estimation may be useful in the assessment of the degree of oxidative stress in pregnancy. Increased intake is thus advocated.

Key Words: Antioxidants, biochemical markers, oxidative stress, pregnancy. [Trop J Obstet Gynaecol, 2004; 21:122-124]

Introduction

Pregnancy is essentially a physiological process, which involves a great number of biochemical changes.[1] Some of these changes that occur in pregnancy cause oxidative stress in the body. Oxidative stress here means an increase in the formation of reactive oxygen species, free radicals and/ or decreased antioxidant defence, and has been recognized as an important feature of many diseases.[2] Most women take iron supplements in order to meet the increased iron requirement during pregnancy[3]. This may predispose them to iron toxicity due to over load. Iron is a transition metal and in excess, may catalyze the formation of reactive oxygen species (ROS).

Free radicals are highly reactive chemical species with an unpaired electron in an atomic or molecular orbital[4]. Radiations, environmental pollutants and drugs are among the many exogenous initiators of free radical generation. In vivo, the most important sources of free radical species are univalent, biochemical redox reactions involving oxygen[5]. Common free radicals in biological systems include superoxide, hydroxyl radicals and other non-radical oxygen derivatives such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Excess levels of ROS can damage proteins and DNA and may destroy antibiotics leading to possible cell injury and death[2].

Antioxidants, on the other hand, are defence biomolecules and mechanisms that combat the effect of free radical damage. Primary antioxidant enzymes include superoxide dismutase (SOD), glutathione peroxidase and catalase. Vitamins C, vitamin E, uric acid, albumin, bilirubin and thiols are classified as non enzymic antioxidants[6]

Oxidative stress occurs when reactive oxygen species (ROS) generation exceeds available antioxidant defences. Pregnancy is accompanied by a high metabolic energy demand and increased oxygen requirement, thus predisposing the body to increased risk of oxidative stress.[7]

Vitamin C is one of the most important water-soluble antioxidants in cells efficiently scavenging a range of reactive oxygen species[8]. By this action, it is able to protect biomembranes and low-density lipoproteins (LDL) from peroxidative damage. Low levels of vitamin C in pregnancy have been suggested to be indicative of the possibility of oxidative stress.[9] Bilirubin and uric acid are also important indicators of oxidative stress.[8,10]

The purpose of the present investigation, therefore, is to evaluate the serum levels of the antioxidants ascorbic acid, uric acid and bilirubin in pregnant subjects and controls and relate same to the levels of oxidative stress in pregnancy.

Subjects and Methods:

The present investigation was carried out on sixty-five (65) pregnant women (uncomplicated singleton pregnancies irrespective of parity), attending the ante-

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natal clinic at the University of Nigeria Teaching Hospital (UNTH) Enugu. Their pregnancies were judged normal based on history, clinical examinations, routine laboratory tests and ultra-sonographic examinations. The pregnant subjects were in their second and third trimesters and within the age range of 20-38 years. The mean age of this group was 29.0 (± 4.19) years. Sixty-five (65) non-pregnant control subjects selected from the medical students, students of the School of Medical Laboratory Technology and staff of the University of Nigeria Teaching Hospital (UNTH) Enugu, were used as control subjects. Here, early pregnancy was ruled out by checking the last menstrual period and samples were taken on the 7th day of their cycles. They were in the same age range (20-38) and social class (class 3- upper middle) as the test subjects, with a mean age of 28.40 (± 5.32) years. Both groups of subjects were selected by simple random selection of lucky dip of yes/no, after informed consent..

For both test and control subjects, fasting venous blood was collected from the ante-cubital vein into sterile plain bottles. Blood samples were allowed to stand for about thirty (30) minutes to clot and then centrifuged at 3,500 rpm for ten (10) minutes. The serum was collected and kept frozen in the refrigerator. Analysis was done within two weeks of sample collection.

Ascorbic acid determination was carried out by the method of Roe and Kenther [11], bilirubin by Powell's method [12] and uric acid estimation by the Phosphotungstic acid method of Brown.[13]

Results

The results obtained from the study showed that in the second trimester of pregnancy, serum ascorbic acid (19.0 ± 2.95 mmol/L) was significantly decreased when compared with control subjects (35.3 ± 8.94 mmol/L), ($P < 0.05$). Also, the serum uric acid level showed a statistically significant decrease in pregnant subjects (2.5 ± 0.48 mg/dl) when compared with the non-pregnant control women (4.9 ± 0.68 mg/dl), ($P < 0.05$). Bilirubin concentration in the serum of the pregnant subjects (0.2 ± 0.18 mg /dl) was similarly significantly lower than that of controls (0.5 ± 0.23 mg/dl), $P < 0.05$ (See Table 1).

Table 1: Comparison of Antioxidant Levels in the Second Trimester (Tests) and Controls:

Antioxidant	*Subjects:n=29 (Test)*	*Controls:n=65*	*P - Value*
Ascorbic acid (mmol/L)	19.0 ± 2.95	35.3 ± 8.94	P < 0.05
Uric acid (mg/dl)	2.5 ± 0.48	4.9 ± 0.68	P < 0.05
Bilirubin (mg/dl)	0.2 ± 0.18	0.5 ± 0.23	P < 0.05

Values are mean ± SD

When the mean values of the anti-oxidants (ascorbic acid, uric acid and bilirubin) at different gestational period (third trimester) were each compared with the non-pregnant control subjects, there were significant decreases ($P < 0.05$ in each case) in the values of all the parameters/antioxidants (Table II).

Table 2: Comparison of the Antioxidant Levels in the Third Trimester (Tests) and Controls:

Antioxidant	*Subjects:n= 36 (Test)*	*Controls:n= 65*	*P-value*
Ascorbic acid (mmol/L)	18.5 ± 2.32	35.3 ± 8.94	P < 0.05
Uric acid (mg/dl)	2.4 ± 0.35	4.9 ± 0.68	P < 0.05
Bilirubin (mg/dl)	0.19 ± 0.17	0.5 ± 0.23	P < 0.05

Values are mean ± SD

Note: Results are expressed in the tables as mean values ± Standard Deviation (SD) and statistical analysis was by the students"t' test method.

Discussion:

Pregnancy is essentially a physiological process requiring a lot of energy and increased oxygen supply. The increased intake and utilization of oxygen results in high levels of oxidative stress.[7] Similarly, relatively more oestriol is produced in pregnancy, leading to increased formation of free radicals, which are capable of mediating tissue damage both in the pregnant woman and the growing fetus.[14]

The present investigation showed statistically significant decreases ($P < 0.05$) each in the serum levels of ascorbic acid, uric acid and bilirubin of pregnant women in the second trimester compared with controls (table 1). There is also a steady decline in the serum levels of these antioxidants with increasing gestational age third trimester (table 2). The second and third trimesters were considered because; most of the pregnant women would not bother to register at the antenatal clinics at the first trimester. The lower levels of these antioxidants during pregnancy may be explained from their utilization as defence mechanisms against reactive oxygen species/free radicals during oxidative stress. They are used in mopping up free radicals and reactive oxygen species generated in high amounts in pregnancy. Other factors that may influence serum antioxidant levels is haemodilution of pregnancy when plasma volume may increase by 40 50 % [15] Ascorbic acid is also actively transferred from mother to fetus and there is increased urinary excretion during pregnancy. The increased urinary excretion is also applicable to uric acid.

The present study, therefore, supports and amplifies the fact that oxidative stress exists in pregnancy. Recent reports have shown that oxidative stress is involved in the aetiology of defective embryonic development[16], pre-term premature rupture of membranes[17], pre-eclampsia[18], recurrent abortion[19], and intra-uterine growth retardation[20]. It is suggested that, since

biological fluids contain the antioxidants ascorbate, urate and bilirubin, their measurements may be used as simple biochemical markers for the assessment of oxidative stress in pregnancy.

In our environment (UNTH Enugu), 300mg/day of ascorbic acid is part of the routine supplements prescribed for our pregnant women. This has been a long time tradition aimed at enhancing iron absorption and assisting other haematinics in red blood cell production. This may not be the case in many hospitals in the third world countries. Despite our prescriptions, the present investigation still showed a statistically significant decrease ($P < 0.05$) in ascorbic acid during pregnancy. Therefore, the need for a substantial increase in ascorbic acid intake during pregnancy is advocated, more so, now that the metabolic role has gone beyond prevention of scurvy. The antioxidant and connective tissue strengthening capabilities of ascorbic acid are now universally accepted by main stream medicine.

The advocated antenatal increase in ascorbic acid supplementation may go a long way in preventing pregnancy complications, thus reducing our high meternal/perinatal mortality and morbidity and 'taking us a step nearer safe motherhood.

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