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Research Article

Assessment of Total Antioxidant Capacity and Lipid Profile among Pregnant Women Attending Ante Natal Clinic in University of Calabar Teaching Hospital, Nigeria

Ogar Francis Awusha[1], Agu Chidozie Elochukwu[1]*, Inaku Kenneth Ogar[2], Nsonwu Augusta[3], Etukudo Maisie[4]
[1]Post-graduate student, Department of medical laboratory science, University of Calabar, Cross river state, Nigeria
[2]Lecturer, Department of chemical pathology and immunology, University of Calabar, Cross river state, Nigeria
[3]Lecturer, Department of medical laboratory science, University of Calabar, Cross river state, Nigeria
[4]Professor, Department of medical laboratory science, University of Calabar, Cross river state, Nigeria

***Corresponding Author:**
Agu Chidozie Elochukwu
Email: chidozieagu@gmail.com

Abstract: Normal pregnancy is associated withchanges in lipid metabolism and high metabolic demand accompanied by elevated tissue oxygen requirements and increased oxidative stress. The present study was carried outto evaluate lipid profile and total antioxidant status in pregnant women at UCTH, Calabar, Nigeria. Serum lipid profile and total antioxidant capacity were estimated in ninety (90) apparently healthy pregnant women aged between 18 and 40 years and thirty non-pregnant controls. Test subjects were divided into three trimesters based on their gestational age. Blood samples were collected from all recruited participants; total antioxidant capacity and lipid profile were analyzed using colorimetric methods. Lipid profile parameters and TAC varied among the various trimesters of pregnancy and in the controls ($P<0.05$). Controls had significantly higher mean values of TAC compared to the 2nd and 3rd-trimester group ($P<0.05$). The first trimesters group had higher TAC and lower mean total cholesterol and LDL-C compared to the 2nd and 3rd-trimester group ($P<0.05$). Third-trimester group recorded higher mean value of TG compared to the 1st trimester and control group ($P<0.05$). Pregnancy and increasing gestational age may be associated with decreased total antioxidant capacity and increased TC, LDL-C and TG.

Keywords: Total antioxidant capacity, pregnancy, total cholesterol, triglycerides, low-density lipoprotein cholesterol.

INTRODUCTION

Scientific evidence has shown that there is a significant underlying relationship between antioxidant activities and pregnancy [1]. The assumption that free radicals can influence thenormal human metabolic state has received substantial scientific support [2]. Elevated reactive oxygen and nitrogen species with depressed antioxidant activity level have been found to be associated with both uncomplicated and complicated pregnancies [3].

Antioxidants are compounds and reactants that dispose of, scavenge, and suppress the formation of reactive oxygen species (ROS) or reactive nitrogen species, which oppose their actions. In other words, an antioxidant is a substance, which prevents the transfer of electrons to and from molecular oxygen, organic molecules, stabilizes organic free radicals and terminates free radical reactions [3].

Oxidants (reactive oxygen species) are normal products of aerobic metabolism called freeradical (not necessarily derived from oxygen or nitrogen) containing one or more unpaired electrons. Reactive oxygen species (ROS) are highly reacting oxidizing agents belonging to the class of free radicals. The most common ROS that has potential implication in pregnancy include;superoxide (O_2) anion, Hydrogen peroxide (H_2O_2), Feroxyl (ROO) and Hydroxyl (OH) [4].

However in pregnancy, redox is increased with associated severe oxidative stress. These factors and the intracellular steps are critical for cell death including mitochondrial dysfunction and importantly the formation of ROS and peroxidation and decreased antioxidant level. Normal pregnancy is associated with high metabolic demand and elevated requirements for tissue oxygen [3]. This result in increased production of reactive oxygen species, as a consequence, antioxidant is hereby proposed as an adjunct therapy for pregnant women [5].

Pregnancy is accompanied by significant variations in maternal lipid metabolism [6]. In early pregnancy, there is increased body fat accumulation associated with both hyperphagia and increased lipogenesis while in late pregnancy there is an accelerated breakdown of fat depots,which plays an

important role in fetal development [6]. A review of theliterature has revealed conflicting observations on normal and abnormal pregnancies [7]. Increase in maternal lipid profile during pregnancy differs with trimester. It has been observed that the concentration of serum total cholesterol, serum triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol in normal pregnant women increased with increasing gestational age [7].

MATERIALS AND METHODS

Selection of subjects

Ninety (90) pregnant women with age ranging from 20-40 years attendingantenatal clinic in the Department of Obstetrics and Gynecology University of Calabar Teaching Hospital (UCTH) were selected for the study. The selection of patients was done with the help of a Gynecologist at the department. Sixty (30) apparently healthy, age-matched women who had given birth to at least one child within the last three years, were selected to serve as the control group. All the subjects gave informed consent to participate in the study. The pregnant women were further grouped based on their duration of pregnancy into first, second and third trimester.

Anthropometric parameters

Anthropometric parameters such as height (m) and weight (kg) were recorded. Weight and height were measured with the subjects wearing light clothing and without shoes.Weight was measured in kilogram using a balanced scale; height was measured in meters using a wall-mounted ruler with the subjects standing with feet together and with head, shoulder, buttocks and heels touching the wall.

Body mass index was calculated as weight in kilogram divided by the square of the height in meters (kg/m^2).

Sample collection

A standard venepuncture method was used to obtain five milliliters (5ml) of blood fromthe entire subject under aseptic conditions. 3milliliters dispensed into a plain container capped, labeled appropriately and allowed to clot at room temperature.

The serum was separated from the red cell by spinning at 3,000 r.p.m. for 5minutes. The supernatant serum obtained was stored frozen at -20^0C until the day of analysis.

Quantitative determination of total antioxidant capacity in the serum using (*Koracevic et al, 2001)*[8] method

Principle: Theassay measuresthe capacity of the serum antioxidants to inhibit the production of thiobarbituric reactive substances (TBARS) from sodium benzoate under the influence of the oxygen radicals derived from Fenton's reaction. Antioxidants from the added serum sample caused suppression of the production of thiobarbituric acid reactive substance (TBARS) and the inhibition of colour development is defined as antioxidant activity which was measured spectrophotometrically at 532nm.

Determination of lipid profile parameters

Total cholesterol and triglyceride were determined using theenzymatic colorimetric method. High-density lipoprotein cholesterol was determined using the precipitation cholesterol enzymatic method; the kits were obtained from EliTech clinical systems (SAS- Zone Industrielle- 61500 SEES France).

Very low-densitylipoprotein-cholesterol concentration was calculated from the triglyceride concentration using the formula;

$$\text{VLDL} = \frac{\text{Triglyceride concentration}}{2.2}$$

Low-density lipoprotein cholesterol concentration was calculated from the total cholesterol concentration, HDL-cholesterol concentration and the triglyceride concentration using the Friedewal *et al.*, formula [12];

$$\text{LDL} - \text{C (mmol/L)} = \frac{\text{Total cholesterol} - \text{HDL - C}}{2.2} + (\text{Triglyceride})$$

Statistical analysis

Statistical analysis was performed using SPSS software version 18 (California Inc.). Data are expressed as mean ± standard deviation. Data between groups were compared using a one-way analysis of variance (ANOVA), followed by post hoc analysis with Tukey's test. Values of $P<0.05$ were considered statistically significant.

RESULTS

Table 1 shows the comparison of anthropometric parameters and blood pressure in various trimesters of pregnancy with the non-pregnant control subjects included in the study. Subjects in third trimester and control subjects had significantly higher mean value of body mass index compared to the first-trimester group ($P<0.05$).

Table 2 shows the comparison of total antioxidant status and lipid profile across the various trimesters of pregnancy and control group. Non-pregnant controls recorded significantly higher mean values of TAC compared to the third and second-trimester groups ($P<0.05$), while the first trimesters group recorded higher mean values of TAC compared to the second and third-trimester group($P<0.05$). Second and third-trimester groups had significantly higher mean values of total cholesterol and LDL-C compared to the first trimester and control group ($P<0.05$).Also, the third-

trimester group had significantly higher mean values of triglyceride compared to the first and second-trimester group (P<0.05).

Table 1: Comparison of Systolic blood pressure, Diastolic blood pressure and Body Mass Index in the various trimesters of pregnancy and the control subjects

Parameters	First trimester (n=30)	Second trimester (n=30)	Third trimester (n=30)	Control (n=60)	F value	P value	Remarks
Age (years)	30.13±4.85	29.87±5.36	29.87±5.30	29.07±5.57	0.23	0.875	No significance
Systolic BP (mmHg)	107.97±14.48	108.30±23.75	114.30±27.08	106.17±7.33	0.96	0.413	No significance
Diastolic BP (mmHg)	70.93±8.50	73.60±10.46	71.90±12.18	73.10±6.38	0.47	0.706	No significance
BMI (kg/m^2)	23.20±2.88	24.90±2.96	26.20±2.82$^{\#}$	25.07±2.02$^{\#}$	6.31	0.001	Significant

Mean ± SD
Significant at P<0.05
Key: $^{\#}$= significantly higher than class I

Table 2: Comparison of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride and TAC in the various trimesters of pregnancy and the control subjects

Parameters	First trimester (n=30)	Second trimester (n=30)	Third trimester (n=30)	Control (n=60)	F value	P value	Remarks
TC (mmol/L)	5.14±0.70	5.86±0.53$^{\alpha\#}$	5.86±0.52$^{\alpha\#}$	5.36±0.41	13.14	0.000	S
HDL (mmol/L)	1.24±0.33	1.28±0.26	1.31±0.22	1.41±0.24*	2.43	0.068	NS
LDL (mmol/L)	3.47±0.60	4.22±0.74$^{\alpha\#}$	4.02±0.50$^{\alpha\#}$	3.47±0.34	10.75	0.000	S
TG (mmol/L)	0.96±0.14	0.97±0.19	1.12±0.14*$^{\#}$	0.95±0.09	8.74	0.000	S
TAC (µmol/L	1563.33±305.67$_{*}^{\beta}$	1126.67±326.65	921.67±145.44	1750±442.17*$^{\beta}$	42.10	0.000	S

Mean ± SD
Significant at P<0.05
Key: $^{\#}$ = significantly higher than class I
***** = significantly higher than class II
$^{\beta}$ = significantly higher than class III
$^{\alpha}$ = significantly higher than control

DISCUSSION

In this study, total antioxidant capacity concentrations were significantly lower in the second and third-trimester group compared to the first trimester and the controls (P<0.05). A possible explanation for this variation is the decreased oxidative stress in the first trimester and control group, thus leading to reduced utilization of the antioxidants

The results of this study clearly showed that total antioxidant status is significantly higher in women with no pregnancy and levels were remarkably decreased as the gestation period increased. These findings are in agreement with that of Toescu *et al*, [9], who reported increased markers of lipid peroxidation as pregnancy progresses. Oxidative stress marker (lipid peroxidation) has been reported to be higher in the second trimester, peaked in third-trimester gestation and decrease after delivery.

Mean total cholesterol and triglyceride concentration varied among the various trimesters of pregnancy and the control, third and second trimesters had significantly higher mean total cholesterol and triglyceride compared to the first trimester and the controls (P<0.05). Possible explanation for this is that during early pregnancy there is an increase in body fat accumulation, associated with both hyperphagia and increased lipogenesis. The increased lipid production during pregnancy is necessary as an energy store to fulfill maternal and fetal metabolic needs while maternal hypertriglyceridemia, especially towards late gestation,

has an important role as a source of triglycerides for milk formation just before parturition [10]. During late pregnancy there is an accelerated breakdown of fat depots, that plays a key role in fetal development. Moreover, using placental transferred fatty acids the fetus benefits from two other products: glycerol and ketone bodies. Although maternal glucose is quantitatively the main substrate crossing the placenta, glycerol is the preferential substrate for maternal gluconeogenesis.

Although maternal cholesterol is an important source of cholesterol for the fetus during early gestation, its importance becomes minimal during late pregnancy, due to the high capacity of fetal tissues to synthesize cholesterol which contributes to the cholesterol pool. This is shown by the marked increase of mean total cholesterol in second and third trimesters. Maternal hypertriglyceridemia is a characteristic feature during pregnancy and corresponds to an accumulation of triglycerides not only in very low-density lipoprotein (VLDL-C) but also in low (LDL-C).

Although triglycerides do not cross the placental barrier, the presence of lipoprotein receptors in the placenta, together with lipoprotein lipase, phospho-lipoproteins A2 and intracellular lipase activities, allows the release to the fetus of polyunsaturated fatty acids transported as triglycerides in maternal plasma lipoproteins. This explains the marked TG seen in second and third trimester where the fetus is almost or fully developed unlike the in first trimester.

Late pregnancy is associated with the formation of susceptible, ox disable particles and an increase in oxidative damage. These biochemical changes may be relevant for the long-term cardiovascular health of women, especially those of high parity who are at high risk for cardiovascular disease like women with diabetes [11].

CONCLUSION

This research work evaluated the levels of total antioxidant capacity and lipid profile parameters in the various trimesters of pregnancy. Results from this study indicate that pregnancy and increasing gestational age is associated with decreased total antioxidant capacity. Also some lipid metabolic indices (TC, LDL-C and TG) increase with the progression of gestational age.

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