



## **Status of Some Antioxidant Micronutrient and Pregnancy Outcomes in Ghanaian Adolescents Attending Antenatal Clinic in Urban (Suntreso) and Rural (Mampong) Hospitals**

**Jessica Ayensu<sup>1</sup>, Anthony Edusei<sup>2</sup>, Ibok Oduro<sup>3</sup> and Christopher Larbie<sup>1\*</sup>**

<sup>1</sup>Department of Biochemistry and Biotechnology, Faculty of Biosciences, College of Science, KNUST, Ghana.

<sup>2</sup>Department of Community Health, School of Medical Sciences, KNUST, Ghana.

<sup>3</sup>Department of Food Science and Technology, Faculty of Biosciences, College of Science, KNUST, Ghana.

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors JA, AE, IO and CL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JA and CL managed the analyses of the study. Author JA managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/EJNFS/2017/24209

**Received 8<sup>th</sup> January 2016**

**Accepted 11<sup>th</sup> July 2017**

**Published 19<sup>th</sup> July 2017**

**Original Research Article**

### **ABSTRACT**

**Aims:** Antioxidants are important in maintaining cellular function in normal pregnancy and are needed for mitigating the effects of oxidative stress. However, there is paucity of information on the importance of antioxidants in pregnant adolescents. This study was therefore aimed at assessing maternal antioxidant micronutrient status and its impact on pregnancy outcomes in Ghanaian pregnant adolescents.

**Study Design:** Prospective Cohort study.

**Place and Duration of Study:** Mampong and Suntreso Government hospitals in the Ashanti Region of Ghana between March and November 2014.

**Methodology:** We included 100 pregnant adolescents aged 11 to 19 years. Two 24 hr recall sessions were used to assess dietary antioxidant micronutrient (Vitamin A, E, C, Zinc and Selenium) intakes of study participants. Biochemical status was assessed by measurement of serum Vitamin A, zinc and selenium using standardized methods. Pregnancy outcomes were obtained from hospital records after parturition.

\*Corresponding author: Email: ekowlarbie@gmail.com;

**Results:** The mean intakes of Vitamin A ( $246.86 \pm 26.80$  mcg/d), E ( $7.32 \pm 0.46$  mg/d) and zinc ( $7.56 \pm 0.42$  mg/d) of participants during the study were below the RDA for the nutrients. The mean serum concentrations of Vitamin A ( $22.64 \pm 1.78$  µg/dl) and zinc ( $137.43 \pm 25.27$ ) were found to be higher than reference values. However, with a mean concentration of  $63.20 \pm 13.58$  µg/dl, serum selenium deficiency was observed in 74% of the participants. The mean birth weight and gestational age of the study population was  $2.89 \pm 0.05$  kg and  $38.23 \pm 1.06$  weeks, respectively (for 43 participants). Twenty-three percent (23%) of the babies were born with low birth weight. Further statistical analysis revealed no association between the antioxidant micronutrient status and birth weight and gestation age.

**Conclusion:** No association was found between the antioxidant micronutrient status and pregnancy outcomes among Ghanaian pregnant adolescents. However, larger epidemiological studies and intervention trials are required to reinforce or refute the beneficial role of antioxidant micronutrient on pregnancy outcomes.

*Keywords: Adolescent; antioxidants; micronutrients; pregnancy outcomes.*

## 1. INTRODUCTION

Normal pregnancy has been proven to be a pro-oxidative stage and oxidative stress has been suggested to play a part in pregnancy-related abnormalities, such as faetal death, preeclampsia, intrauterine growth restriction (IUGR) and preterm delivery [1-3]. Antioxidants are important in maintaining cellular function in normal pregnancy and are needed for mitigating the effects of oxidative stress. The antioxidant defense system of the body is influenced by micronutrients with distinguished antioxidant properties such as vitamin C, vitamin E, vitamin A, selenium, zinc, copper and manganese and antioxidant functions of these nutrients during gestation has taken center stage in most recent studies [4-8]. Outcomes of these studies have however been inconsistent, increasing the need for more research in this field. Results of an observational Study conducted by Kiondo et al. [9] in Uganda revealed a positive relation between maternal plasma vitamin C levels and preeclampsia in pregnant women in Uganda. Rayman et al. [10] also found low serum selenium at the end of the first trimester to be associated with preterm delivery. These studies and others highlight the roles of antioxidant micronutrients in preventing abnormalities in pregnancies although several supplementation studies have not supported the protective roles. For instance, Bastani et al. [11] found no significant difference between the APGAR scores, birth weight and the incidence of preeclampsia in pregnant women who received 400IU of vitamin E from the 14th week of gestation till parturition and a control group who received no supplements. In another studies Weissgerber et al. [12] found no positive effect of vitamin E and C supplementation from the 9th to

16th weeks of gestation till parturition on preeclampsia prevention. Their results revealed that supplementation increased the risk of preeclampsia and the late onset of preeclampsia by three folds in Hispanic women. Results of another supplementation study showed no effect of selenium supplementation ( $60$  µg/dL) from 12 to 14 weeks of gestation until delivery on preeclampsia risk among English women of low selenium status [5].

Most of these studies have been carried out in developed countries and in pregnant adults, placing little emphasis on pregnant adolescents who are at a higher risk for adverse pregnancy outcomes owing to anatomical immaturity, competition for nutrients between mother and foetus, inadequate intakes of essential nutrients, poor eating habits, economic constrains, poor nutritional knowledge and high cost of maternal health care [10,13-15]. This study was therefore aimed at assessing maternal antioxidant micronutrient status and its impact on pregnancy outcomes in Ghanaian pregnant adolescents.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

Pregnant adolescents were recruited from two government hospitals in the Ashanti region of Ghana; Mampong and Suntreso Government hospitals. The Mampong Government hospital is situated in the Mampong municipal (rural) while Suntreso hospital is in the Kumasi metropolis (urban). The subjects were 100 pregnant adolescents, aged 11 to 19 years (the adolescent age), living in the Ashanti region of Ghana. They were categorized as being urban or rural dwellers upon recruitment.

## 2.2 Sampling Techniques

The sample size was calculated using the Cochrane's formula based on a prevalence 6% [16] at 5% marginal error. A total of 100 participants were involved in the study. The population for the study was stratified into two categories. These categories represented adolescents residing in urban and rural areas. Respondents from the rural and urban areas were recruited from the Mampong and Suntreso Government hospitals in the Ashanti region, respectively. The convenience sampling technique was used to select pregnant adolescents attending ANCs at these hospitals for the study. The Mampong Government hospital was chosen because it is the district hospital and referral hospital in the Mampong Metropolis. The Suntreso Government hospital is also one of the well-equipped hospitals in Kumasi and attracts clients from various parts of the Kumasi municipality. These hospitals also have well organized reproductive centers which provide antenatal care to pregnant adolescents. The stratification was made to allow comparison between the two groups. Pregnant expectant teenage women with singleton pregnancies aged 11 to 19 years, who did not have any medical complications and attended antenatal clinic (ANC) in the two facilities, were recruited for the study. Pregnant teenagers with complications during the pregnancy and were multiparous were excluded from the study.

## 2.3 Dietary Assessment

Two 24 Hour dietary recall sessions (one on a week-day and one on a week-end) were used to assess the daily antioxidant micronutrient intakes of pregnant adolescents. In an interview, participants were asked to recall food and beverage intake of previous 24 hours. Common household measures and food models were used to aid respondents to estimate the amount of foods and beverages consumed. However, no considerations were given to recall days and intervals between recall days. Samples of commonly consumed foods by respondents were purchased from food vendors. These were weighed with a food scale (Mettler Toledo- Viber SM 30) and used to prepare a conversion factor for all the venter foods. The conversion table was used to estimate the quantity of foods consumed by respondents from the 24 hour recalls in grams. Antioxidant micronutrient contents of foods consumed by respondents were determined using a Nutrient Analysis

Template (University of Ghana, Food Science and Nutrition Department and the West African Food Composition Table [17]. This template provides the nutrient content of raw foods, convenient foods and composite dishes usually consumed in Ghana. The West African Food Composition Table also provided the nutrient content of raw and cooked food ingredients. Nutrient intake was compared with the recommended dietary allowance for pregnant adolescents by the National Institute of Health.

## 2.4 Biochemical Assessments

At recruitment, 5 ml of venous blood was collected from subjects into gel tubes. The blood samples were centrifuged at 3000 rpm for 5 minutes for isolation of serum. The serum samples were frozen at -22°C until they were ready to be analyzed. Vitamin A was analyzed using High Performance Liquid Chromatography (HPLC) [18] while the minerals (Zinc and Selenium) were analysed with Atomic Absorption Spectroscopy (AAS) [19] at the Nutrition Laboratory at the Noguchi Memorial Institute for Medical Research and the Ecological Laboratory at the University of Ghana respectively.

### 2.4.1 Serum retinol analysis

The Shimadzu HPLC equipment (components - Pump - Shimadzu - LC- 6A, Recorder - Shimadzu - C -R5A, Detector - Shimadzu - SPD - 6A, Column - Reversed phase (ODS Hypersil 5 µm, 2.1x50 mm) and Injector - Rhyodyne 1745) was used for the serum retinol analysis by the method described by Kim & Quadro [18]. One hundred and twenty microliters (120 µg) of serum was pipetted into a 2 ml Eppendorf tube and deproteinised with 120 µl of methanol. The solution was vortexed for 30 seconds. Extraction of retinol was done with 500 µl of hexane by vortexing for 2 minutes. Samples were centrifuged at 10,000 rpm for 2 minutes; 250 µl of the supernatant (hexane layer) was evaporated to dryness under a stream of nitrogen gas. This was reconstituted with 120 µl of methanol and 20 µl was injected into the pump for analysis. Serum retinol concentration was measured at a wave length of 325 nm [17]. Persons with retinol concentrations below 20 µg/dl (0.7 µmol/l) were considered to be deficient of Vitamin A [20].

### 2.4.2 Serum zinc and selenium analysis

The Perkin Elmer Pinaacle 900T AAS equipment was used for the serum selenium and zinc

analysis by the method described by Severi et al. [19]. Preparation of analyte was done by pipetting 0.2 ml of the serum into test tubes with addition of 9.8 ml deionized water to make up 10 ml, at dilution ratio of 1:50. All samples and standards were analyzed in triplicates. Subjects with serum zinc concentrations below 70 µg/dL were considered to be deficient of zinc [19]. Subjects with serum selenium concentrations below 89 µg/L (range 89- 114 µg/L) were considered to be deficient of selenium [21].

## 2.5 Pregnancy Outcomes

Pregnancy outcomes were obtained from hospital records after parturition and categorized as term, preterm, normal birth weight, low birth weight, fetal deaths, spontaneous abortions, abortion and perinatal deaths.

## 2.6 Statistical Analysis

Statistical analysis was performed using SPSS version 16 and Microsoft Excel 2010. Descriptive statistics were computed for nutrient and demographic variables. Independent- sample T-Test was used to determine whether differences were present among two age groups or location and ANOVA used to determine differences among the three trimesters. Frequency distributions were calculated to determine the portion of the sample with nutrient intakes relative to the dietary reference intakes. Continuous variables were summarised as means with their standard deviations. The

Pearson Correlation was used to determine relationships between variables. The level of significance applied to statistical tests was 95%.

## 3. RESULTS AND DISCUSSION

A total of 100 pregnant adolescents aged 11 to 19 years were enrolled in the study from March to November, 2014. The mean age of the study population was 17.8± 1.7 years. Of the 100 adolescents, 42% and 58% were recruited from Kumasi and Mampong Government hospitals respectively. Other socio-demographic and obstetric characteristics of participants are shown in Table 1.

The mean intakes of vitamin A (246.86±26.80), zinc (7.56±0.42) and vitamin E (7.32±0.46) of the pregnant adolescents were found to be below the RDA and over 60% of the participants had intakes below the reference values. Mean intakes of Vitamin C (123.69±7.9) and selenium (65.94±3.04) exceeded or met the RDA for pregnant adolescents (Table 2). These results show that a large portion of the participants are at risk of having inadequate daily intakes of vitamins A, vitamin E and zinc. Mean intake of vitamin A recorded in this study is lower than that obtained by Poboick et al. [22]; Oguntona & Akinyele [23]; Giddens et al. [24] among Guamanian (1,093 mcg), Nigerian (2305.5 mcg) and American (1,053 mcg) pregnant adolescents respectively. As in this study, other researchers report low intakes of vitamin E by pregnant adolescents [11,25,26].

**Table 1. Distribution of the demographic characteristics of respondents (N=100)**

Characteristics	Total (%)	Kumasi (%)	Mampong (%)
<b>Age</b>			
<15	5	3	2
15-19	95	39	56
Total	100	42	58
<b>Trimester</b>			
First	21	6	15
Second	45	22	23
Third	34	14	20
<b>Educational status</b>			
Basic school	79	33	46
Senior high school	16	8	8
None	5	1	4
<b>Occupation</b>			
Student	22	9	13
Trader	29	13	16
Unemployed	49	20	29

**Table 2. Dietary antioxidant micronutrient intakes based on percent of the RDA**

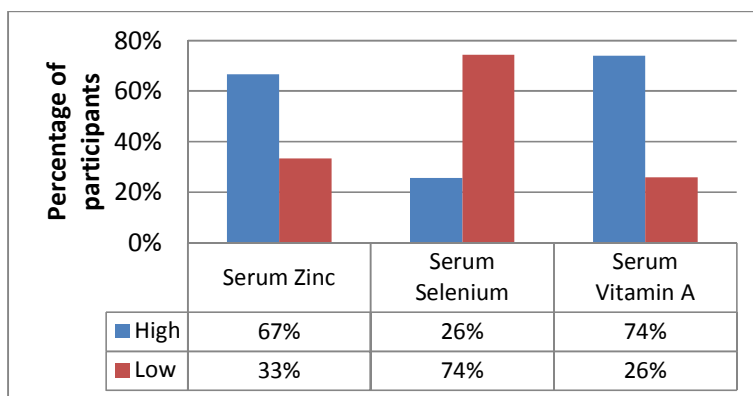
Nutrient	RDA	Intake Mean± SEM	Percent RDA	Percent sample N (%)		
				<75% RDA	75% to < 100% RDA	≥ 100% RDA
Vitamin A	770 mcg/d	246.86±26.80	32	93 (93)	4 (4)	3 (3)
Vitamin C	85 mg/d	123.69±7.9	145	24 (24)	10 (10)	66 (66)
Vitamin E	15 mg/d	7.32±0.46	48.8	63 (63)	30 (30)	7 (7)
Selenium	65 mcg/d	65.94±3.04	101	30 (30)	28 (28)	42 (42)
Zinc	12 mg/d	7.56±0.42	63	74 (74)	19 (19)	7 (7)

The distribution of participants according to the serum concentrations of vitamin A, zinc and selenium are presented in Fig. 1. The mean serum concentration of selenium was 63.20 µg/l± 13.58. Almost 74.4% (n=74) of the study subjects had deficient serum selenium levels. The mean serum vitamin A and zinc concentrations were 22.64 µg/dl ± 1.78 and 137.43± 25.27 respectively. The mean serum concentrations of vitamin A and zinc are an indication of Vitamin A and zinc sufficiency in pregnant adolescents who participated in the study.

Data on pregnancy outcomes was available for only 43 participants; the remaining participants either gave birth at home or in other clinics which were beyond the reach of the investigators. The mean gestational age for the study sample was 38.23±1.06 weeks and the mean birth weight for all births was 2.89±0.50 kg. Nearly 49% of the index infants had birth weights under the mean birth weight (2.89 kg). Ten (23.3%) of the infants had low birth weights and 4.9% were preterm. Majority (88.4%) of the participants went through normal deliveries. Further statistical analysis revealed no significant association between the antioxidant micronutrient status of participants and birth outcomes (Table 3). The findings of this

study add support to the body of literature showing that maternal antioxidant micronutrient status was not associated with pregnancy outcomes [5,11,12]. Rayman et al. [5] found no beneficial effect of selenium supplementation on pre-eclampsia risk in women who received 60 µg of selenium daily from the 12 week of gestation till parturition. The results however contradict findings of other observational and experimental studies that concluded that maternal antioxidant micronutrient status was associated with pregnancy outcomes [6,10,15,27,28]. Olang et al. [6], found serum vitamin A levels to be inversely related to gestational age, implying that, an increase in the serum vitamin A levels will result in decreased gestational age or increase the risk for preterm delivery and vice versa.

Although reports on the effects of antioxidant micronutrients on pregnancy outcomes are inconclusive, the lack of associations in this study could be attributed to the fact that data on pregnancy outcomes was available for only 43 participants. Again, the use of a 2- day 24 Hour Recall instead of the recommended 3-day 24 Hour Recall could either have underestimated or overestimated the dietary intakes of participants.

**Fig. 1. Serum antioxidant micronutrient status of pregnant adolescents**

**Table 3. Relationship between antioxidant micronutrient status of pregnant adolescents and pregnancy outcomes**

Antioxidant micronutrients	Pregnancy outcomes	
	Birth weight, r (p-value)	Gestational age, r (p-value)
Dietary vitamin A	-.03 (.85)	.30 (.87)
Dietary vitamin C	.26 (.13)	.26 (.12)
Dietary vitamin E	-.01 (.60)	.12 (.50)
Dietary selenium	-.10 (.55)	.03 (.88)
Dietary zinc	.10 (.57)	.10 (.55)
Serum vitamin A	.11 (.79)	-.32 (.38)
Serum selenium	-.28 (.36)	-.10 (.75)
Serum zinc	.13 (.65)	-.01 (.98)

*Data presented are the correlation coefficients (r) and p-values for the association between antioxidant micronutrient status and pregnancy outcomes*

#### 4. CONCLUSION

Findings from this study revealed that pregnant adolescents had inadequate intake of vitamin A, vitamin E and zinc. Pregnant adolescents also had high serum concentrations of selenium and vitamin C. This study shows that antioxidant micronutrient status has no significant impact on pregnancy outcomes among pregnant adolescents. Future studies must put measures in place to adequately monitor all participants and obtain all data needed.

#### ETHICAL CONSIDERATION

Ethical approval was sought and obtained from the Committee on Human Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (CHRPE/KNUST) for the commencement of this research. Approval Number: CHRPE/AP/132/14. A written permission to carry out the research was obtained from the medical superintendents of the hospitals from where data was collected. Verbal or written consent was also sought from each pregnant adolescent and from guardians who were with their wards at the antenatal (ANC) for voluntary participation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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