Hematological Significance of Vitamin A, C and E Bi-combination in Pregnancy

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ABSTRACT: The present study evaluates the correlation between antioxidant vitamins A, C and E bi-combinations and basic hematological parameters in pregnancy. 70 adult female Wister rats weighing between 250- 300g were procured and grouped into 2 control groups treated with distilled H_2O and vehicle tween-80 respectively and three cohorts (I, II and III) with four sub-groups (n = 5). After mating with male Wister rats, pregnancy was confirmed on the 6th day. Administration of test agents began on the 7th day. Cohort group I received a combination of vitamin A and C, group II; vitamin A and E and group III; vitamin C and E respectively at four different doses for 11 days. Blood samples were collected 24hrs after completion of treatment and analyzed for Hemoglobin concentration (Hb), white blood cell (WBC) and differential count. Results revealed a significant increase ($p<0.05$) in Hemoglobin concentration in the vitamin A, C and E bi- combination treated groups. This increase was dose dependent. WBC count was significantly lower ($p<0.05$) in experimental groups as compared to the control ($8300.00 \pm 836.66/mm^3$). However, increase in dose brought about increase in WBC count in the experimental groups. Polymorph and lymphocyte count presented mixed results compared to control values following alteration in dosage. Antioxidant vitamins A, C and E bi-combinations posses synergetic potentials and appear promising in pregnancy.
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Key words: *Hematological parameters*, *Antioxidant Vitamins*, Bi- c*ombination* therapy

INTRODUCTION

Pregnancy is characterized by physiological and metabolic changes which are the result of altered maternal nutritional requirements, superimposed foetal metabolism and its needs, pregnancy-related physiological adaptations and re-tailored hormonal homeostasis (Wondmikun, 2004). All these alterations increase the demand of the mother for essential nutrients and the supply of adequate nutrients to pregnant women is one of the basic requirements for proper embryonic development, and normal foetal and maternal health (Wondmikun, 2004). To a large extent, the hematological profile reflects general Health (WHO, 2004) and many studies have identified the hematological profile of pregnant women as one of the factors affecting pregnancy and its outcome (Klebanoff et al., 1991; Allen, 2000; Bothwell and Charlton, 1981). Low hemoglobin concentration is the most widely identified hematological abnormality (CDCP, 1998) and is associated with adverse pregnancy outcome (Klebanoff et al., 1991; Allen, 2000; Meng et al., 1991). The nutritional status of pregnant women is therefore a concern particularly in regions where nutritional deficiency is a public health problem even when some nutritional supplements are cheap and available.

On the other hand, vitamin supplementation is known to impact significant benefit in disease prevention and treatment and has been widely accepted as a measure of control of micro nutrient deficiencies (Ekaidem et al., 2006). Numerous epidemiological evidences support the beneficial role of dietary antioxidant vitamins (Donaldson, 1982; Hodis *et al*., 1995; Stephens *et al*., 1996), while some studies also questioned their beneficial role (Gaziano *et al*., 1995; Zhang *et al*., 1997). Previous study by Iribhogbe et al (2010) on pregnant rats revealed that antioxidant vitamin A, C and E monotherapy caused a dose dependent alteration in hematological profile. In this study, hematological values were not significantly increased by antioxidant vitamin C and E monotherapy except for vitamin A monotherapy (Iribhogbe et al., 2010). Interestingly, several researches on antioxidant vitamins combination have been a topic of discussion and there had been remarkable reports. To this end, previous study have shown that the administration of the antioxidants under consideration in varying combination significantly reduced serum Na^+ concentration, caused alteration in serum lipids and alteration in the hyper-hepatic state of pregnancy . This may be significant in the management of hypertension in pregnancy (Iribhogbe et al., 2011a), beneficial in the management of pregnancy related hyperlipidemic states (Iribhogbe et al., 2011b) and also beneficial in the hyper-hepatic state of pregnancy (Iribhogbe et al., 2011c). The question now is what is the likely effect

of bi-combination of these antioxidant vitamins on hematological parameters which are considered as health indices and of diagnostic significance in clinical evaluation of the state of health? It is therefore the aim of this study to investigate the significance of antioxidant vitamin A, C and E bi-combination on basic hematological parameters in pregnancy.

MATERIALS AND METHODS

Animals:

Seventy adult female Wister albino rats (250- 300 g) were obtained from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma, between August and October 2009 and were housed in Physiology Lab 1 of the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State. Nigeria. They were assigned into three test groups (I, II and III) made up of four sub- groups (n = 5 rats each). The control group was made up of a negative control and a vehicle/ tween 80 group with 5 rats each. The animals were allowed to acclimatize for 2 weeks and fed *ad libitum* with tap water and pelleted feeds purchased from Bendel feeds and flour meal Ewu, Nigeria Limited. Two adult male Wister albino rats were introduced into each sub-group to allow for mating for 6 days after which the male animals were removed from the cage. Pregnancy was confirmed using the palpation method as described by Agematsu *et al*. (1983) and vaginal smear microscopy method typified by the presence of scanty epithelial cells and leucocytes (Long and Evans, 1922; Daly and Kramer, 1998). Administration of the different vitamins combination (see table 1) began on the 7th day using orogastric tubes and syringes to minimize loss of the test substances (Ejebe *et al*., 2009) between the hours of 08.00 am and 10.00 am daily and this lasted for a period of 11 days.

Vitamin preparation:

Vitamin A, C and E were purchased from Clarion Medical Pharmaceuticals Nigeria Limited and Tween 80 vehicle from Sigma Pharmaceuticals Limited. 200 mg of the powdered form of vitamin C was dissolved in 10 mL of distilled water and the appropriate dose per kg was prepared for administration. Vitamin A (25,000 IU equivalent to 6 mg retinal and E, 100 mg) was dissolved in 0.2 mL of tween 80 and water in a ratio of 0.2:0.2:9.6. Group I received Vitamin A + C combination at 4 different doses. Group II received vitamin A + E while group III received vitamin E+ C at 4 different doses, respectively (Table 1).

Samples collection:

Twenty-four hours after the last administration was carried out, the animals were sacrificed after inhalation of chloroform. Cardiac and jugular vein puncture were used to collect blood samples into sterilized test tubes containing K3 EDTA as anticoagulant.

Analysis of the Hematological Parameters:

The White Blood Cells (WBC) and the differential count were estimated using the Improved Neubauer counting chamber as described by Baker et al., (1990). The Hemoglobin (Hb) concentration was determined by the Cyanmeth-haemoglobin method also as described by Baker et al (1990).

Data analysis:

The mean ± standard error of mean was determined and test samples were compared using student's t-test performed using SPSS version 17 soft ware. The significance level was set at $p < 0.05$. Results were presented in suitable tables.

RESULT

A comparison of hemoglobin concentration measured during bi- combination of antioxidant vitamins supplementation in early pregnancy in Wister rats is shown in Table 2. There was an increase in hemoglobin concentration in the entire groups and this increase was dose dependent. Compared to the control (13.80 ± 0.47 g/dl), bi-combination of vitamin C and E was not statistically different ($p> 0.05$) while vitamin A and C and A and E bicombination presented a significant increase ($p < 0.05$). However, the action of vitamin A and C was more potent. Table 3, revealed the total WBC count in treatment and control groups fed with different bi-combination of antioxidant vitamins. Statistical analysis revealed that there was a significant ($p<0.05$) reduction in treatment groups when compared with control (8300.00 ± 836.66 mm^3). However, WBC increased with increasing dose in the entire treatment groups. As shown in Table 4 and 5, bi- combination of Vitamin A and C, and C and E presented a similar pattern in the differential WBC count (polymorhonuclear and lymphocyte count respectively). Vitamin A and E presented an increased polymorhonuclear count with increasing dose while the lymphocyte count was observed to be reduced. Although mixed results were presented, comparatively with the control (38.00 ± 6.52 %), significant decrease ($p<0.05$) in polymorhonuclear count with increasing dose was present in the entire treatment groups. Similarly, lymphocyte count followed the same pattern of mixed results with a significant increase ($p>0.05$) when compared to the control (62.00 ± 6.52 %).

Table 1: Treatment administered to different groups (n = 5 rats per group)

Group	Treatment
Control Vehicle:	Normal feed + Distilled water 1 mL Normal feed + Tween 801 mL
Vitamin A+C	1. Normal feed + Vehicle + Dist H2O + Vit A 0.6 mg/kg + Vit C 200 mg/kg 2. Normal feed + Vehicle + Dist H20 + Vit A 0.7 mg/kg + Vit C 250 mg/kg 3. Normal feed + Vehicle + Dist H2O + Vit A 0.8 mg/kg + Vit C 300 mg/kg 4. Normal feed + Vehicle + Dist H2O + Vit A 1.0 mg/kg + Vit C 400 mg/kg
Vitamin A+E	1. Normal feed + Vehicle + Dist H2O + Vit A 0.6 mg/kg + Vit E 16.4mg/kg 2. Normal feed + Vehicle + Dist H2O + Vit A0.7 mg/kg + Vit E 18.4mg/kg 3. Normal feed + Vehicle + Dist H2O + Vit A 0.8 mg/kg + Vit E 19.4mg/kg 4. Normal feed + Vehicle + Dist H2O + Vit A 1.0 mg/kg + Vit E 22.4mg/kg
Vitamin E+C	1. Normal feed + Vehicle + Dist H2O + Vit E 16.4 mg/kg + Vit C 200 mg/kg 2. Normal feed + Vehicle + Dist H2O + Vit E 18.4 mg/kg + Vit C 250mg/kg 3. Normal feed + Vehicle + Dist H2O + Vit E 19.4 mg/kg + Vit C 300mg/kg 4. Normal feed + Vehicle + Dist H2O + Vit E 22.4mg/Kg + Vit C 400mg/kg

Table 2: Comparative changes in Hemoglobin concentration (g/dl) in control and antioxidant bi-combination treatment groups

Treatments	Vitamin A + C	Vitamin A + E	Vitamin C + E
Control	13.80 ± 0.47^{a}	13.80 ± 0.47^{a}	13.80 ± 0.47^{a}
Tween 80	13.40 ± 0.31^{a}	13.40 ± 0.31^{a}	13.40 ± 0.31^{a}
T1	13.88 ± 0.48^{a}	13.84 ± 0.38^{a}	$13.52 \pm .55^{a}$
T2	14.84 ± 0.32^{b}	14.18 ± 0.34^{a}	13.88 ± 0.22^{a}
T3	14.86 ± 0.44^{b}	14.46 ± 0.48^{b}	14.08 ± 0.56^{a}
T4	15.08 ± 0.36^{b}	14.72 ± 0.33^{b}	14.16 ± 0.43^{a}

Values are mean ± SD (g/dl) for fiver rats each; mean of the same column followed by different letters differ significantly ($p < 0.05$) compared with control. (Key: T = treatments).

Table 3: Comparative changes in White blood cell count (mm^3) in control and antioxidant bi-combination treatment groups

Treatments	Vitamin A + C	Vitamin A + E	Vitamin C + E
Control	8300.00 ± 836.66^{a}	8300.00 ± 836.66^{a}	8300.00 ± 836.66^{a}
Tween 80	8050.00 ± 1328.53^{a}	8050.00 ± 1328.53^{a}	8050.00 ± 1328.53^{a}
T1	4550.00 ± 503.74^{b}	3750.00 ± 672.68^{b}	3050.00 ± 467.71^{b}
T2	5600.00 ± 407.74^{b}	5200.00 ± 331.66^{b}	4750.00 ± 448.61^{b}
T3	5850.00 ± 755.81^{b}	5600.00 ± 302.08^{b}	5500.00 ± 308.22^{b}
T4	6350.00 ± 329.77^{b}	5750.00 ± 220.79^{b}	5850.00 ± 364.01^{b}

Values are mean ± SD ($/mm^3$) for fiver rats; mean of the same column followed by different letters differ significantly ($p \leq 0.05$). (Key: T = treatments).

Table 4: Comparative changes in Polymorphs count (%) in control and antioxidant bi-combination treatment groups

Treatments	Vitamin A + C	Vitamin A + E	Vitamin C + E
Control	38.00 ± 6.52[a]	38.00 ± 6.52[a]	38.00 ± 6.52[a]
Tween 80	36.00 ± 2.74[a]	36.00 ± 2.74[a]	36.00 ± 2.74[a]
T1	41.40 ± 3.58[a]	26.60 ± 3.71[b]	31.60 ± 3.36[b]
T2	38.40 ± 4.34[a]	26.80 ±2.496[b]	29.60 ± 3.58[b]
T3	37.20 ± 4.44[a]	29.80 ± 3.90[b]	29.60 ± 3.36[b]
T4	30.00 ± 3.74[b]	30.20 ± 4.36[b]	27.80 ± 4.71[b]

Values are mean ± SD (%) for fiver rats; mean of the same column followed by different letters differ significantly ($p \leq 0.05$). (Key: T = treatments).

Table 5: Comparative changes in Lymphocytes count (%) in control and antioxidant bi-combination treatment groups

Treatments	Vitamin A + C	Vitamin A + E	Vitamin C + E
Control	62.00 ± 6.52[a]	62.00 ± 6.52[a]	62.00 ± 6.52[a]
Tween 80	64.00 ± 2.74[a]	64.00 ± 2.74[a]	64.00 ± 2.74[a]
T1	58.60 ± 3.58[a]	73.40 ± 3.71[b]	68.40 ± 3.36[b]
T2	61.60 ± 4.34[a]	73.20 ±2.496[b]	70.40 ± 3.58[b]
T3	62.80 ± 4.44[a]	70.20 ± 3.90[b]	70.40 ± 3.36[b]
T4	70.00 ± 3.74[b]	70.0 ± 4.36[b]	72.20 ± 4.71[b]

Values are mean ± SD (%) for fiver rats; mean of the same column followed by different letters differ significantly ($p \leq 0.05$). (Key: T = treatments).

DISCUSSION

Normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which results in increased oxidative stress and antioxidant defenses (Knapen, 1999). It is well established that lipid peroxides, such as thiobarbituric acid reactive substances increase significantly in the maternal circulation (Arikan *et al.,* 2001) and Zachara *et al., (1993) and* Qanungo and Mukherjea, (2000) reported glutathione peroxidase and superoxide dismutase activities to be reduced during the second trimester of pregnancy in humans. Morris *et al* (1998) reported high circulating levels of lipid peroxides in normal pregnancy and preeclampsia.

Although low hemoglobin concentration is the most widely identified hematological abnormality (CDCP, 1998) and is associated with adverse pregnancy outcome (Klebanoff et al., 1991; Allen, 2000; Meng et al., 1991). Unlike our previous study where antioxidant vitamin monotherapy presented significant reduction in hemoglobin concentration except for vitamin A (Iribhogbe et al., 2010), the present study revealed an increase in hemoglobin concentration with bi-combination of antioxidant vitamins which was more potent with vitamin A and C bicombination. Previous study revealed that vitamin C and E monotherapy showed no improvement in hematological values (Iribhogbe et al., 2010). Vitamin A supplementation has previously been shown to improve hematological status, and increase mobilization of iron stores (Mejia and Chew, 1988). This suggests a potential synergistic effect of antioxidant vitamin bi-combinations. Knowing that anemia during pregnancy is a common medical problem that have deleterious effects on the mother and fetus evidenced by increased maternal morbidity and mortality, intrauterine growth retardation, poor weight gain, premature labor, preterm delivery and perinatal morbidity and mortality (Bothwell, 2000), coupled with the fact that normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which results in increased oxidative stress and antioxidant defenses (Knapen, 1999). Consequently bi-combination of antioxidant vitamins during this period of life for sustaining mother and fostering the growth and maintenance of fetus is suggested. Interestingly, McDowell (1989) reported that the combination of antioxidant vitamins and minerals showed greater antioxidant ability against oxidative damage.

Similarly, unlike in the study of antioxidant vitamin monotherapy where WBC was decreasing

with increasing doses (Iribhogbe et al., 2010), WBC increased with increasing doses of antioxidant vitamin bi-combination in the present study, suggesting antimicrobial potential of vitamin combinations. Interestingly, the combination of vitamins A and E and C and E has been shown to suppress parasitaemia (Umar et al, 2008). Although increase in WBC was observed with increasing dose of bi- combination of antioxidant vitamins, the increase was not higher than the control value. An increasingly higher dose may be necessary to elevate WBC count in antioxidant vitamin combination. However, it has been shown that the effect of antioxidant in infections with different species of trypanosomes was attributed to the protection of membrane and cellular components against oxidative species by the vitamins (Umar et al, 2008). As in previous study of antioxidant vitamins monotherapy were there was a significant reduction in polymorhonuclear count with increased dose and a non significant increase in lymphocyte count (Iribhogbe et al., 2010), bi- combination of Vitamin A and C, A and E, and C and E presented a similar pattern. While Vitamin A and E presented an increased but significant reduction in polymorhonuclear count with increasing dose, lymphocyte count was observed to be reduced. Although mixed results were presented, comparatively with the control (38.00 ± 6.52 %), a significant decrease ($p<0.05$) in polymorhonuclear count was present in the entire treatment group. Similarly, lymphocyte count followed the same pattern of mixed results with a significant increase ($p>0.05$) when compared to the control (62.00 ± 6.52 %).

Several experimental and clinical studies suggest an interaction between micro nutrients. Smith (1980), Solomon and Russell (1980) and Christian and West (1998) showed zinc and vitamin A interaction in treatment of vitamin A deficiency. Other researchers revealed that vitamin A supplementation alone failed to revert vitamin A deficiency (Rahman et al, 2002). Yakoob et al (2010) has reported widespread maternal vitamin and mineral deficiencies and further recommend it logical to consider supplementation with multiple micronutrient preparations in pregnancy. There is therefore a need to consider multivitamins and minerals in pregnant state based on the findings of this study. The clinical benefits of such an approach over single-nutrient supplements are unclear (Yakoob et al, 2010).

CONCLUSION

There is a synergistic influence of antioxidant vitamin A, C and E bi-combination on hematological profile in pregnancy. Hence, we advocate the use of multiple- vitamin combination therapy in pregnancy.

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