Antioxidants And Hematological Profile In Pregnancy

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ABSTRACT: This study aims to investigate the effect of supplementation with vitamin A, C and E on hematological profile in pregnancy using Wister Albino rats. 85 adult female rats were procured from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma. They were divided into 5 groups; a control group A, vehicle group B, vitamin A treated group C, vitamin C treated group D and vitamin E treated group E all having 5 animals each respectively. After pregnancy was confirmed, the control group A received distilled water, the vehicle group B received Tween-80 while the test groups C, D and E were dosed oro-gastrically with vitamin A, vitamin C and vitamin E at five different doses for eleven days respectively. Blood samples were collected 24hrs after completion of treatment and analyzed for Hemoglobin concentration (Hb), complete white blood cell (WBC) and differential count. Results were analyzed using SPSS version 17. Student t test was performed and a p value < 0.05 was considered significant. Results revealed that at a very low dose, vitamin A, C and E produced no significant change in Hemoglobin concentration. However, a dose dependent change was observed in the hematological profile. WBC count was lower in the experimental group when compared with control. Polymorph count was significantly reduced in the test groups ($p < 0.05$) while lymphocyte count remained unchanged when compared with control ($p > 0.05$). Vitamin A, C and E cause a dose dependent alteration in hematological profile in pregnancy.

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INTRODUCTION

In general, clinical surveys and hospital records indicate that malnutrition is severe among vulnerable groups, which include infants, preschool children, pregnant women and lactating mothers (Atinmo, 1983; Paramjit et al., 2004). Majority of the citizens in developing countries do not have access to balanced diet. Various researches conducted in developing countries attest to the fact that even the diet they consume are frequently not enough to meet even their energy requirements (Cole, 1987; Singh et al., 1989; Minghelli et al., 1990). Vitamin supplementation is known to impart significant benefit in terms of disease prevention and treatment and has been widely accepted as a Measure of control of micro nutrient deficiencies (Ekaidem et al., 2006). On the other hand, vitamin supplementation during some specific state may be deleterious to health and hence defeat the very purpose of its administration (VIC, 1997). Hematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Patrick-Iwuanyanwu et al., 2010). Thus, this study seeks to investigate the changes in hematological parameters in early pregnancy associated with the use of these supplements.

MATERIALS AND METHODS

Animals:

Eighty five (85) adult female Wister albino rats weighing 200-300 g were procured from the Animal House of the College of Medicine, Ambrose Alli University, Ekpoma between July and September 2009 and were housed in a stainless steel cage with plastic bottom grid and a wire screen top in a well-aerated laboratory located in the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were randomly allocated into 5 groups; a control group A (n=5), vehicle group B (n=5), vitamin A treated group C (n=5 per sub-group), vitamin C treated group D (n=5 per sub-group) and

vitamin E treated group E (n=5 per subgroup). They were fed *ad libitum* with tap water and pellated feeds purchased from Bendel Feeds and Flour Meal Limited, Ewu, Edo State. The animals were allowed to acclimatize for 2 weeks, after which two (2) male Wister albino rats were introduced into each group to allow for mating which lasted for 6days. The male animals were removed from the cage after the 6th day and pregnancy was confirmed in the female rats using the palpation method and vaginal smear microscopy method. From the 7th day, administration of the different vitamins at 5 different doses (Vitamin A; 0.6mg/kg, 4mg/kg, 10mg/kg, 15mg/kg and 25mg/kg. Vitamin C; 100mg/kg, 200mg/kg, 350mg/kg, 500mg/kg and 600mg/kg. Vitamin E; 15mg/kg, 40mg/kg, 100mg/kg, 150mg/kg and 200mg/kg) was commenced for each group using orogastric tubes and syringes. This lasted for a period of 11 days. The dosing was conducted between the hours of 08.00 am and 10.00am daily.

Vitamins and dose preparation:

Vitamin A, C and E were purchased from Clarion Medical Pharmaceuticals Nigeria Limited. Tween 80 vehicle was purchased from Sigma Pharmaceuticals Limited. 200mg of the powdered form of vitamin C was dissolved in 10mls of distilled water and the appropriate dose per kg was prepared for administration. Vitamin A (25,000 IU equivalent to 6mg retinal and E, 100mg) was dissolved in 0.2ml of tween 80 and water in a ratio of 0.2:0.2:9.6. Group C, D and E received Vitamin A, C and E respectively (see table 1 for details of dose administration).

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 K_3 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.

RESULTS

There was a significant reduction in hemoglobin concentration ($p<0.05$) in the vitamin C and E treated groups. However, a different pattern was observed in the vitamin A treated group, which revealed a dose dependent increase in hemoglobin concentration, which peaked (21.80±0.80g/dl) after the administration of the 5th dose (Table 1). This increase is significant ($p<0.05$) when compared with control (13.80±0.47g/dl). A sustained decrease was observed in the total WBC count in the entire vitamin treated groups, this however was dose dependent and significant when compared with control ($p<0.05$). As shown in Table 3, the differential WBC count showed a significantly sustained decrease in polymorhonuclear count as dose increased in the entire micronutrient group when compared with control ($p<0.05$). This was not the case with the lymphocyte count, which showed no significant difference when Compared with control ($p>0.05$).

Table 1: Mean Hemoglobin concentration in micronutrient treated groups

Groups	Dose (mg/kg)	Hb g/dl
A (control)	1ml	13.80±0.47
B (vehicle)	1ml	13.40±0.31
C (vitamin A)	0.6	14.00±0.89
	4.0	∗15.00±0.39
	10.0	∗15.00±0.41
	15.0	∗19.40±0.39
	25.0	∗21.80±0.80
D (vitamin C)	100	13.50±0.45
	200.0	∗11.80±1.57
	350.0	∗11.50±0.68
	500.0	∗11.00±1.25
	600.0	∗10.30±0.99
E (vitamin E)	15.0	13.50±0.47
	40.0	∗11.40±1.22
	100.0	∗11.00±1.34
	150.0	∗8.70±0.76
	200.0	∗7.00±0.80

Results expressed as mean ± SEM. ∗ = p<0.05 when compared with control

Table 2: Mean WBC count in different treatment groups

Groups	Dose (mg/kg)	WBC (/mm^3)
A (control)	1ml	8300.0±1360.61
B (vehicle)	1ml	8050.0±1328.53
C (vitamin A)	0.6	∗5100.0±912.41
	4.0	∗3950.0±508.67
	10.0	∗3000.0±540.84
	15.0	∗2950.0±778.62
	25.0	∗2850.0±863.13
D (vitamin C)	100	∗3000.0±755.81
	200.0	∗2950.0±643.23
	350.0	∗2950.0±707.99
	500.0	∗1800.0±627.50
	600.0	∗1650.0±826.89
E (vitamin E)	15.0	∗3900.0±752.50
	40.0	∗3400.0±451.39
	100.0	∗3300.0±337.27
	150.0	∗2850.0±117.04
	200.0	∗2050.0±737.39

Results expressed as mean ± SEM. ∗ = p<0.05 when compared with control

Table 3: Mean WBC differential count in the treatment groups

Groups	Dose (mg/kg)	Polymorphs (%)	Lymphocytes (%)
A (control)	1ml	38.0 ±4.30	80.0±4.30
B (vehicle)	1ml	35.0 ±1.22	82.0±1.22
C (vitamin A)	0.6	34.0 ±2.74	84.0±2.74
	4.0	∗22.0 ±1.22	78.0±1.22
	10.0	∗20.0 ±2.00	80.0±2.00
	15.0	∗17.0 ±2.55	83.0±2.55
	25.0	∗13.0 ±6.44	79.0±6.44
D (vitamin C)	100	∗26.0±1.87	84.0±1.87
	200.0	∗19.0±4.30	79.0±4.30
	350.0	∗19.0±6.60	74.0±6.60
	500.0	∗16.0±6.52	77.0±6.52
	600.0	∗16.0±3.32	78.0±3.32
E (vitamin E)			
	15.0	∗24.0±5.05	79.0±5.05
	40.0	∗22.0±4.64	85.0±4.64
	100.0	∗21.0±6.28	77.0±6.28
	150.0	∗19.0±3.61	81.0±3.61
	200.0	∗17.0±5.92	80.0±5.92

Results expressed as mean ± Results expressed as mean ± SEM. ∗ = p<0.05 when compared with control

DISCUSSION

The hematological profile of an individual to a large extent reflects their 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, 200; Bothwell and Charlton, 1981). The most commonly mentioned hematological indices are the indicators of hemoglobin concentration. 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). From our study, vitamin A supplementation in pregnancy at a very low dose appears harmless. It was observed that Hemoglobin concentration in the pregnant rats increased as dose increased consecutively. This was in concordance with the findings of Chawla and Puri, (1994) and NIN, (1989). Following the administration of vitamin A at consecutively increasing doses, total WBC count was observed to significantly reduce, this was also observed in other micronutrient groups. Vitamin A supplementation has previously been shown to improve hematological status, and is thought to increase mobilization of iron stores (Mejia and Chew, 1988). This finding was supported by this study. However, the study of Fawzi et al., (2007), Christian et al., (2003) and Ramakrishnan et al., (2004) revealed an opposite effect. Vitamin A supplementation has also been shown to have little or no effect in maternal and fetal outcome in anemia (Fawzi et al., 1993; Fawzi et al., 2003). In our study, vitamin C supplementation in pregnancy significantly decreased hemoglobin concentration, total WBC count and differential polymorph count while differential lymphocyte count remained unchanged with increasing doses. This can be explained by the study of Eteng et al., (2003) who reported that vitamin C produced a slight increase in serum Na+ and K+ level. Interestingly, increased plasma sodium ion inhibits rennin release with consequent withdrawal of angiotensin II (Jackson, 1984). Furthermore, K+ stimulates aldosterone production. Angiotensin II and aldosterone are reported to expand extracellular fluid volume and blood volume Guyton and Hall, (2006). Thus increase blood volume with no increase in cellular components will result in a reduction in hematological indices.

Vitamin C has also been reported to contribute to pre-eclampsia in pregnant women who were "self-medicating" with high doses of the vitamin in the mistaken belief that it did them good (Marsh, 2006). Furthermore, report reveals that like vitamin K, vitamin C can be pro oxidative (Jackson, 1984) and kill healthy cells (Sakagami, 2000) this may explain the reduced WBC and differential polymorph count. High dose vitamin C decreases serum vitamin B12 levels (Herbert, 1994; Simon, 1999) and increase activation of mutagenic HCA from food (Hsieh, 1997). Vitamin C

can enhance tumors (Agus, 1999) and increase oxidative free iron level (Simon, 1994; Attiek, 1999) when used far above therapeutic dose range.

Also in a study conducted in rats, during the first month of pregnancy, high doses of vitamin C may suppress the production of progesterone from the corpus luteum (Ovcharov and Todorov, 1974). Progesterone is known to be involved in hematopeoisis, thus decreased progesterone levels may account for the reduction in blood indices following vitamin C administration.

High dose vitamin C may be involved in abortion and miscarriages. Guyton and Hall, (2006) reported that progesterone is involved in providing nutrient for fertilized ovum and in reducing uterine smooth muscle excitability and motility. From our study, vitamin E did not appear to improve Hb concentration, WBC count and polymorph count when compared with control. It produced a significant reduction in all the blood parameters assessed; however there was no significant change in differential lymphocyte count. Vitamin E concentration has been shown to rise throughout pregnancy (Leonard; 1972) and its deficiency is relatively uncommon in pregnancy (Dostalova, 1982; Kaminetzky et al., 1973).Thus, in well-nourished women adequate vitamin E is consumed in the diet and supplementation is not required. In two studies, vitamin E supplementation at 500 and 600mg daily during the last 2 months of pregnancy did not produce values significantly different from controls in the erythrocyte hemolytic test (Gyorgy et al., 1952; Mino and Nishino, 1973). Research has previously suggested that vitamin E, an antioxidant, has a protective effect against miscarriage and pre-eclampsia, particularly when taken with high doses of vitamin C. But two recent studies in London and Australia, published in The Lancet and the New England Journal of Medicine, suggest that the reverse may be true (Marsh, 2006). Furthermore, excess vitamin E has been shown to cause bleeding in the brain (The Alpha-Tocopherol, Beta-carotene Cancer Prevention Study Group, 1994) and impairs immune system reactivity, which can cause cancer or arthritis (Herbert, 1997). Cells contain more vitamin E leading to an unbalanced antioxidant status, and an increasing susceptibility to oxidative radicals (Grammatico, 1998). This may contribute to the global reduction in hematological parameters observed in our study. Multivitamins designed for pregnancy is a good idea but this is promoting intake in mega doses due to the inherent believe that it is beneficial to maternal and child health. Optimizing the pre and inter pregnancy micronutritional status of women particularly in low resource setting could be extremely beneficial (Bartley et al., 2005, Owens and Fall, 2008) and may lead to improved pregnancy outcome (Ruchi, 2009) as also revealed by this study. At larger doses, the beneficial effect may not be guaranteed due to the potential risk of toxicity.

CONCLUSION

In most studies where vitamins are proven to be beneficial they are used in combination and at lower doses. Although these micronutrients have some benefit in pregnancy, we recommend that hematological parameters should be monitored during their use, particularly following high dose administration in pregnancy.

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