Effects of Antioxidant Vitamin Combination on Electrolyte Status in Pregnancy

[1]O.I. Iribhogbe, [1]J.E. Emordi, [2]B.O. Idonije, [3]U. Akpamu, [3]E.O. Nwoke and [1]A. Aigbiremolen
[1]Department of Pharmacology and Therapeutics,
[2]Department of Chemical Pathology,
[3]Department of Physiology, College of Medicine, Ambrose Alli University,
Ekpoma, Edo State, Nigeria

Abstract: It is a known fact that hypertension is associated with pregnancy. Several studies have reported a link between hypertension and a shift in serum electrolyte concentration. This study is designed to investigate the effect of antioxidant vitamin A, C and E bi- combination on electrolyte status in pregnancy. In a bid to investigate the above objective, varying combination of antioxidant vitamin A, C and E were administered to 70 pregnant Wister albino rats. These were assigned into two control groups treated with distilled H_2O and vehicle- tween-80 respectively and three cohorts (I, II and III) with four sub-groups each (n = 5). Beginning from the 7th day, test group I received a varying dose combination of vitamin A+C, group II vitamin A+E, and group III vitamin C+E respectively for 11 days. Results revealed a non significant reduction (p>0.05) in serum Na^+ and Ca^{2+}, and a significant change p<0.05) in serum Cl^- and K^+ following Vit A+C administration when compared with control. The group treated with Vit A+E combination presented a non significant change in serum Na^+, Cl^-, K^+ and Ca^{2+} level. Vit C+E treated group presented a significant reduction (p<0.05) in serum Na^+, K^+ and Ca^{2+} with no alteration in serum Cl^-. In conclusion, antioxidant vitamin A, C and E bi-combination ameliorates the reported high Na^+ concentration in pregnancy and may be of significance as adjuvants in the management of hypertension in pregnancy. However, the importance of further research cannot be overemphasized.

Key words: Antioxidants, bi-combination, electrolytes, hypertension, pregnancy, synergistic, vitamins

INTRODUCTION

Hormonal imbalance leading to altered lipid profile in serum is attributed to be the prime factor in etiopathogenesis of Pregnancy-Induced Hypertension (PIH) (Sahu *et al.*, 2009). The association of hypertension in pregnancy is well documented as Kamath (2006), reported hypertensive disorders to be common medical complications of pregnancy with a reported incidence of about 10% of first pregnancies and 20 to 25% of women with chronic hypertension. A shift in serum electrolyte in essential hypertension have been acknowledged. Electrolytes are known to be responsible for the osmolarity of the internal environment and their imbalance originates serious illnesses (Coppo, 2001). While some studies have questioned the beneficial role of antioxidant vitamins (Gaziano *et al.*, 1995; Zhang *et al.*, 1997), numerous epidemiological evidences support the beneficial role of these vitamins (Donaldson, 1982; Hodis, 1995; Stephens *et al.*, 1996).

Interestingly, our previous study on the effect of antioxidant vitamin A, C and E mono-therapy on electrolyte status in early pregnancy published in the American Journal of Biochemistry and Molecular Biology, 2011, reported no alteration of serum Na^+ but revealed that vitamin C and E acting alone can produce hypercalcemia and hyperkalemia. Of significance, is the study of McDowell (1989) who reported that the combination of antioxidant vitamins and minerals showed greater antioxidant ability against oxidative damage. Available data has attached some importance to the use of several different regimen in combination. The veracity of synergistic impact led to the postulation that antioxidant vitamin combination therapy may be important in managing dyselectrolemic states in pregnancy. It is in view of the benefit attached to vitamin combination that this study was carried out to ascertain the pattern of electrolyte alteration in pregnancy following supplementation with vitamin A, C and E bi- combination using Albino- Wister rats.

MATERIALS AND METHODS

Experimental animals: Seventy adult female Wister albino rats weighing (225-300 g) were obtained from the Animal House, College of Medicine, Ambrose Alli

Corresponding Author: Dr. O.I. Iribhogbe, Department of Pharmacology and Therapeutics, College of Medicine, Ambrose Alli University, P.M.B. 14. Ekpoma, Edo State, Nigeria. Tel: +2348065794437

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

Group	Treatment
Control	Negative Control: Normal feed + Distilled water 1ml
	Vehicle: Normal feed + Tween 80 1ml
Test group 1 (Vitamin A+C)	1. Normal feed + Vehicle + Dist H2O + Vit A 0.6mg/kg + Vit C 200 mg/kg
	2. Normal feed + Vehicle + Dist H20 + Vit A 0.7mg/kg + Vit C 250 mg/kg
	3. Normal feed + Vehicle + Dist H2O + Vit A 0.8mg/kg + Vit C 300 mg/kg
	4. Normal feed + Vehicle + Dist H2O + Vit A 1.0mg/kg + Vit C 400 mg/kg
Test group 2 (Vitamin A+E)	1. Normal feed + Vehicle + Dist H2O + Vit A 0.6mg/kg + Vit E 16.4 mg/kg
	2. Normal feed + Vehicle + Dist H20 + Vit A0.7mg/kg + Vit E 18.4 mg/kg
	3. Normal feed + Vehicle + Dist H2O + Vit A 0.8mg/kg + Vit E 19.4 mg/kg
	4. Normal feed + Vehicle + Dist H2O + Vit A 1.0mg/kg + Vit E 22.4 mg/kg
Test group 3 (Vitamin E+C)	1. Normal feed + Vehicle + Dist H20 + Vit E 16.4mg + Vit C 200 mg/kg
	2. Normal feed + Vehicle + Dist H20 + Vit E 18.4mg + Vit C 250 mg/kg
	3. Normal feed + Vehicle +Dist H20 + Vit E 19.4mg/kg+ Vit C 300 mg/kg
	4. Normal feed+ Vehicle + Dist H20 + Vit E 22.4mg/Kg+Vit C 400 mg/kg

Table 2. Electrolytes profile of pregnant rats treated with vitamin A and C combination

Group	Na	K	Cl	Ca
Control	139.80±5.81	8.60±0.54	99.00±5.39	0.66±0.02
Tween 80	141.00±1.58	9.70±0.23*	98.00±2.45	0.68±0.02
T1	141.60±3.91	8.60±0.37	100.8±2.28	0.69±0.06
T2	140.40±3.85	7.94±0.18*	103.4±2.97*	0.68±0.02
T2	138.80±2.68	7.54±0.17*	107.20±1.48*	0.66±0.01
T4	136.60±3.65	6.96±0.25*	111.80±3.56*	0.65±0.02

Electrolyte parameters are in mmol/L; Values are mean±SD of five rats; *: level of significance (p<0.05) when compared with control

University, Ekpoma between August and October 2009. They were housed in a stainless steel cage with plastic bottom grid and a wire screen top in physiology Lab II in the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were assigned into five groups; a control group (n = 5), vehicle group (n = 5) and three test groups (I, II and III) made up of four sub-groups each (n = 5). They were fed ad libitum with tap water and pellated feeds purchased from Bendel feeds and flour meal Ewu, Nigeria Limited and allowed to acclimatize for 2 weeks. After which two male Wister albino rats were introduced into each group to allow for mating. The animals were allowed to mate for 6 days after which the male animals were removed from the cage. Pregnancy was confirmed using the palpation method (Agematsu *et al*., 1983) and vaginal smear microscopy method (Long and Evans, 1922; Daly and Kramer, 1998). From the 7th day, administration of the different Vitamin combinations began using orogastric tubes and syringes to minimize the loss of test substance (Ejebe *et al*., 2009) and lasted a period of 11 days. The administrations were conducted between the hours of 08.00 am and 10.00 am daily.

Vitamin preparation and administration: 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 10mls 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. Table 1 for the doses administrated to the test groups.

Sample 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 lithium heparin as anticoagulant.

Electrolytes analysis: Collected blood sample were immediately sent to the biochemical laboratory for analysis. Determination of serum K^+, Na^+, Ca^{2+}, and Cl^- were analyzed using standard methods as described by Tsalev and Zaprianov (1983).

Data analysis: The mean±standard deviation (X ± SD) and one-way ANOVA (LSD) statistical test was performed using SPSS version 17 soft ware with the significance level set at p<0.05 and results were presented with suitable tables.

RESULTS

Table 2, showed the concentration of the serum electrolyte in control and experimental groups following the administration of the combination of Vit A+C. A non significant decrease (p>0.05) in serum Na^+ and Ca^{2+} from the control value of 139.80±5.81 and 0.66±0.02 mmol/L, respectively were observed as dose increases. While serum Cl^- level was increasing as the dose of Vit A+C combination increase, this increase became significant (p< 0.05) from the 2nd to 4th dosing. Serum K^+ was decreased and was significantly (p<0.05) reduced from the 2nd to 4th treatment.

Statistical analysis showed that there was no significant difference (p<0.05) between control and

Table 3: Electrolyte profile of pregnant rats treated with vitamin A and E combination

Group	Na	K	Cl	Ca
Control	139.80±5.81	8.60±0.54	99.00±5.39	0.66±0.02
Tween 80	141.00±1.58	9.70±0.23*	98.00±2.45	0.68±0.02
T1	140.80±2.86	8.50±0.32	100.20±6.69	0.65±0.03
T2	139.60±2.41	8.76±0.17	101.00±4.30	0.67±0.02
T2	138.80±1.64	8.84±0.25	103.80±7.46	0.68±0.05
T4	137.00±1.58	9.00±0.38	103.20±3.35	0.69±0.02

Electrolyte parameters are in mmol/L; Values are mean±SD of five rats; *: level of significance ($p<0.05$) when compared with control

Table 4: Electrolyte profile of pregnant rats treated with vitamin C and E combination

Group	Na	K	Cl	Ca
Control	139.80±5.81	8.60±0.54	99.00±5.39	0.66±0.02
Tween 80	141.00±1.58	9.70±0.23*	98.00±2.45	0.68±0.02
T1	136.20±3.11	8.78±0.29	100.20±6.61	0.69±0.04
T2	134.00±2.74*	8.30±0.25	99.00±5.79	0.65±0.04
T2	131.60±4.98*	7.70±0.20*	100.20±2.17	0.61±0.04*
T4	129.80±3.19*	7.30±0.28*	103.20±5.07	0.56±0.04*

Electrolyte parameters are in mmol/L; Values are mean±SD of five rats; *: level of significance ($p<0.05$) when compared with control

treatment values of serum Na^+, K^+, Cl^- and Ca^{2+} in all the groups administered the different experimental doses of Vit A+E (see table 3).

As shown in Table 4, following vitamin C+E administration, there was an increase in serum Cl⁻ levels which was not significantly different with control (99.00±5.39mmol/L). A significant reduction ($p<0.05$) in serum concentration of Na^+, K^+ and Ca^{2+} was observed.

DISCUSSION

This study confirms the spectrum of changes in the serum concentration of electrolyte during pregnancy. Supporting this finding is the study of Knight *et al*. (1994) who reported that pregnancy is characterized by physiological and metabolic changes which alter maternal biochemical and hematological parameters resulting in an increase or decrease in the normal non-pregnant values. Increase in blood hormone (Van-Thiel *et al*., 1987; Kase *et al*., 1991) and plasma volume expansion (Cheek *et al*., 1985) are no doubt the cause of this shift from normal non- pregnant values in pregnant conditions. These adjustments of adaptive mechanism ensure protection of the developing fetus against any adverse effect that accompanies changes in these parameters (Hytten and pitten 1963). Nutrient requirement during pregnancy are therefore altered by these changes which occur in the mother. Thus, agreeing with Longo (1981) that the supply of adequate nutrients for optimum nutritional needs of the mother is one of the essential requirements for positive pregnancy outcome.

The present investigation revealed that the administration of antioxidant vitamin A, C and E bi-combination caused alteration in electrolyte profile in pregnancy which is associated with elevated amount of electrolyte production, this is indicative of synergism between antioxidant vitamin A, C and E bi- combinations. Serum Cl⁻ was however not affected by antioxidant vitamin bi-combination. Na^+, K^+ and Ca^{2+} were more favourably altered following vitamin C+E combination

which was more potent than vitamin A+C and vitamin A+E combination. Serum Na^+ was significantly reduced, suggesting that antioxidant vitamin combinations may ameliorate hypertension in pregnancy. To the best of our knowledge, information on the effect of vitamin combination on electrolyte status in pregnancy is lacking. However, intake of ß-carotene along with intake of total fiber, vitamin A, vitamin C, iron and magnesium has been reported to have an inverse relation with diastolic blood pressure (Tillotson *et al*., 1997). Short-term oral high-doses of zinc, vitamin C, ß-carotene and alpha-tocopherol have lowered blood pressure, possibly via increased availability of nitric oxide (Galley *et al*., 1997). The precise cause of hypertension in pregnancy is not yet completely understood. According to Guyton and Hall (2006), many physiologists believe that it is due to thickening of the glomerular membranes perhaps caused by an auto-immune process, which reduces the glomerular capillary filtration coefficient and rate of fluid filtration from the glomeruli into the renal tubules; damage to endothelial cells caused by release of a toxic factor from the placenta; or impaired formation of nitric oxide, which causes vasoconstriction of the renal arterioles and increased tubular reabsorption of salt and water. Interestingly, pregnancy is known to affect the endocrine (Banaczek, 1995), cardiovascular (DHawan *et al*., 2005), electrolyte concentration and blood pressure (Obembe and Antai, 2008) and other system especially the renal system since it displays hypertrophy of functions, altered homeostasis and hemodynamic leakage of some substances as well as exaggerated response to posture (Korda, 1987). Also, normal pregnancy have be reported to be accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which results in increased oxidative stress and antioxidant defenses (Knapen *et al*., 1999).

Obembe and Antai (2008) reported pregnancy to be expected to waste K^+ because pregnant women eat and excrete normal quantities of Na^+ yet have a high aldosterone and other mineralocorticoids. This is in

contrast to the non pregnant subjects who are resistant to kaliuresis provoked by combination of exogenous mineralocorticoids and a high sodium intake (Ehrlich and Lindheimer, 1972). This is contrary to the findings of Beausejour *et al*. (2003), who reported that pregnant rats can not handle Na^+ load. The ability to conserve K^+ in the face of high concentration of sodium may be due to high levels of progesterone associated with pregnancy, a view that is not accepted by all (Brown *et al*., 1986). Studies by Obembe *et al*., (2003) found increase in GFR in pregnancy irrespective of parity with the greatest GFR recording in the 2nd trimester. The expansion of plasma volume during pregnancy results in a decrease in plasma protein concentration (Goodlin *et al*., 1983). This decrease in plasma protein is accompanied by an increase in the urinary excretion of amino acids, which amounts to as much as 2 g/day compared to 0.5 g/day in the non-pregnant state (Hytten and Cheyne, 1972). During pregnancy, serum concentrations of the water-soluble vitamins decrease as a result of hemodilution (Hytten 1980, Rivers and Devine 1975). This may be the reason why combination with vitamin C presented a more favourable outcome probably due to it deficit. Several studies have reported the role of vitamin C in reducing high blood pressure (Ness *et al*., 1997; Moran *et al*., 1993; Duffy *et al*., 1999). Chen *et al*. (2002), examined the relation between serum vitamins A, C and E, carotene and ß-carotene levels and blood pressure among men and women (20 years of age) in the US population indicating that antioxidant vitamins may be important in the underlying cause and prevention of hypertension. Direct relationship between plasma volume expansion and birth weight has been reported (Pirani *et al*., 1973). In addition, poor pregnancy outcome is associated with lack of adequate plasma volume expansion (Goodlin *et al*., 1981, Hays *et al*., 1985). Studies with pregnant hypertensive women did not show any relationship between severity of the disease and electrolyte concentration and no difference was found in biochemical tests of renal efficiency between primigravidae and multiparous pregnancies (Banaczek, 1995). MacDonald and Good (1972) found a decreased K^+ and Na^+ concentrations from 10 – 20 weeks, in multiparous but not in primigravidae, with both showing significant increase in K^+ concentration between 28-37 weeks with no change in Na^+ or Cl^-. Green and Halton (1987) found the handling of Na^+, K^+ and Cl^- to be the same in pregnant conscious rats. Many nephrologists believe in the possibility that prolonged period of increased single nephron filtration as in grandmultiparity may exert potentially damaging effect on the glomerulus especially the hyperfiltration and increased glomerular blood pressure (Hostether *et al*., 1982).

Increasing intake of fruits, vegetables and cereals in the diet is currently advocated as a measure to control hypertension (Appel *et al*., 1997; John *et al*., 2002). The DASH (Dietary Approaches to Stop Hypertension) diet, which is rich in vegetables, fruits and low-fat dairy products and reduced sodium intake (below the current recommendation of 100 mmol per day) has been found to lower blood pressure substantially (Sacks *et al*., 2001). The favorable effect of fruits and vegetables on hypertension suggests a possible role of micronutrients and antioxidants available from fruits and vegetables in reducing high blood pressure; as fruits and vegetables are rich sources of antioxidants such as vitamins. Considerable evidence is available from epidemiological observations, intervention trials and studies on experimental animals showing the influence of various dietary components on blood pressure. Most of these studies have been carried out on non-pregnant populations. Our result from antioxidant vitamin bi-combination indicate that bi- combination of vitamin A, C, and E may reduce the risk of hypertension associated with pregnancy since serum Na was significantly reduced. In a randomized double-blind crossover design placebo-controlled study on 21 hypertensives and 17 normotensives, a high dose supplementation of zinc and vitamin C along with alpha-tocopherol and beta-carotene for eight weeks resulted in a fall of SBP in both hypertensives as well as normotensives (Galley *et al*., 1997). Negative correlation of plasma vitamin C and SBP and DBP has been well-documented (Ness *et al*., 1997; Moran *et al*., 1993; Duffy *et al*., 1999). Antioxidant vitamin combination may be an important component in therapeutic reduction in blood pressure, and in the maintenance of low blood pressure.

CONCLUSION

Conclusively, the present finding of this study on antioxidant vitamin combination indicate that bi-combination of vitamin A, C, and E may reduce the risk of hypertension associated with pregnancy since serum Na was significantly reduced. Although, major findings of our study are based on murine model study, further interventional studies are needed to investigate bi-combination of antioxidant vitamins. The importance of further prospective clinical trials using these antioxidant vitamin combinations in addition to supplementation with other micro- nutrients deficient in pregnancy cannot be overemphasized. Till then, antioxidant vitamin A, C and E bi-combination possess an important nutritional therapeutic potential in the amelioration of hypertension in pregnancy via electrolyte modulation as evidenced by our study.

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AUTHORS' CONTRIBUTIONS

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