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RESEARCH PAPER OPEN ACCESS

INFLUENCE OF ANTIOXIDANT VITAMINS ON IRON AND HAEMATOLOGICAL INDICATORS IN PRE-SCHOOL CHILDREN

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ABSTRACT

Vitamins A and E are considered to be important antioxidants and their deficiencies remain independent risk factors for anemia among children. In the present study, sub-clinical vitamins A and E concentrations and haematological parameters were assessed among preschool children from rural areas in Bhubaneswar Tehsil, Odisha. A sample of forty-six children aged upto 5 years from both sexes was included. The mean vitamin A (retinol), and vitamin E (α-tocopherol) concentrations were 34.56±23.0 µg/dl and 645.3±5.32 µg/dl respectively. Vitaimin A deficiency (<20 µg/dl) was 34.8%, while vitamin E deficiency (tocopherol <500 µg /dl) was 43.5%. Children with deficiency of vitamins A and E had significantly low mean hemoglobin (p=0.014 and 0.0001 respectively) and hematocrit (p=0.042 and 0.0001 respectively) values as compared to the non-deficient ones. Red blood cell (RBC) count was found to be significantly lower in vitamin E deficient children (p=0.0004). Serum ferritin levels were found to be significantly high in children deficient with both vitamin A and E followed by vitamin E or vitamin A alone (p=0.005). The results show that levels of vitamins A and E greatly influence iron storage (ferritin) and mobilization (hemoglobin, hematocrit and RBC count) in children suggesting the need for supplementation of these vitamins.

KEY WORDS:
Retinol, α-tocopherol, haematological parameters, ferritin

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INTRODUCTION

Micronutrient deficiencies are now recognized as an important contributor to the global burden of disease. Vitamins are micronutrients required in very small amounts (micrograms to milligrams) to maintain the fundamental functions of the body. Deficiencies of iron, iodine, and vitamin A are widely prevalent and can affect the mental development and learning ability of children (Stuijvenberg et al., 1999). Deficiencies in vitamin A and iron are important public health problems in most developing countries and other micronutrient deficiencies that also include vitamin E. "Antioxidant nutrients" is a term that covers a wide variety of dietary components including vitamins. Antioxidants are the molecules that neutralize harmful free radicals produced through a chain of oxidative reactions (Joseph et al., 2010). Vitamin E, particularly α-tocopherol, functions in vivo as a lipid soluble, chain breaking antioxidant (Ingold et al., 1986; Burton et al., 1983a) and it is a potent peroxyl radical scavenger (Burton et al., 1983b). Among the well documented lytic actions of vitamin A (retinol), red cell haemolysis have been investigated extensively (Krishnamurthy and Kartha, 1973; Lucy, 1970). Several studies in humans and experimental animals have shown that there is an interaction between vitamin A and iron nutrition and metabolism (Hodges et al., 1978; Donoghue et al., 1981). Treatment of iron-deficient patients with a combination of iron and vitamins such as A, C, and E proved effective in normalizing the oxidative stress (Gadjeva et al., 2005).

Vitamin A deficiency continues to be a major health problem in India and has far-reaching consequences on growth, development, and health, especially in children ranging from potentially blinding xerophthalmia to increased risks of infection and mortality (Sommer and West, 1996; Rahmathullah et al., 1990). Although clinical vitamin A deficiency (VAD) in India has declined considerably, subclinical VAD (low retinol concentration) affect more than half of under-5 children. In Odisha, VAD is a significant public health problem. The median blood vitamin A levels (retinol) among preschool children is 18.1 µg/dl, which is less than the assigned value of 20µg/dl for defining VAD in children by WHO (NNMB, 2006). These types of estimates of vitamin A status are based predominantly on dietary or biochemical data, particularly plasma retinol (de Pee and West, 1996). Vitamin E deficiency (VED) occur secondary to fat malabsorption because vitamin E absorption requires biliary and pancreatic secretions. Children with protein energy malnutrition concomitant with VED are shown to have neurological abnormalities (Kalra et al., 1998). Dietary inadequacy of vitamin E will lead to overt VED if the α-tocopherol levels in target tissues (e.g., peripheral nerves) become depleted. Thus, children all through have been the susceptible population in which VED has been observed (Meydani et al., 2005). Anemia is reported to be an early classic symptom of VED in children (Farrell et al., 1977). Although anaemia is a major public health problem among preschool children in India including the state of Odisha, no data exists in establishing its association with VED. Since coexistence of

iron, vitamin A and vitamin E deficiencies are considered as independent risk factors for anemia among children, an attempt has been made to characterize individual and conjoint effects of VAD and VED on hematological variables.

MATERIAL AND METHODS

Pre-school children (upto the age of 5 years) were selected from five villages under Chandaka Gram Panchayat of Bhubaneswar Tehsil, Odisha. The study was approved by institutional ethical committee and informed consent was obtained from the parents of each child after the study objective was explained to them. Confirmation of a child's age was made with the mother with the help of a local-events calendar and from Anganwadi record followed by discussion of the child's feeding practices and frequency of food consumption. Body weight and height measurements of the children were taken according to the recommended procedures of the WHO (2006).

Five hundred microliter of blood sample was collected from each child in EDTA-containing evacuated tubes with the use of disposable single-use needles (Dispovan, India) from a total of 46 children whose parents agreed for drawing venous blood. Each sample vial was wrapped in aluminum foil to protect from light for estimation of vitamin A and E. Red cell indices such as hemoglobin (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), granulocyte (GRA), monocyte (MON) and lymphocyte (LYM) counts were measured from whole blood on automated MS4 cell counter. The remaining blood was centrifuged at 1800 rpm for 5 min at 40C in cold centrifuge to separate plasma into 3 different polypropylene tubes and stored in -200C for subsequent analyses for vitamin A, vitamin E and ferritin. Plasma ferritin was measured by enzyme linked immunosorbent assay (ELISA) using UBI MAGIWEL Ferritin Quantitative test system as per instruction given by manufacturer. Plasma levels of retinol and α-tocopherol (the primary form of vitamin E) were measured simultaneously with high-performance liquid chromatography (HPLC) system (Shimadzu LC-20A) following the method of Siong and Choo, 1999. In brief, 50 µl of plasma was added with ethanol and internal standard retinyl acetate and tocopherol acetate along with 400µl of petroleum ether and mixed well. Petroleum ether layer was separated after centrifugation and evaporated to dryness under a stream of nitrogen and the residue was dissolved in 100µl of methanol. Finally, 20µl samples were injected to HPLC using a C-18 Bondclone 10µm ODS column. Samples were eluted with methanol:water (95:5) maintaining a flow rate of 1 ml/min at a detector wavelength 326 nm up-to 10min and 295 nm up-to 25 min. Quantification of retinol and tocopherol was done by comparing the peak areas of standards (retinol and tocopherol) and internal standards (Sigma-Aldrich) respectively to calculate the recovery of each sample concentrations. All procedures were performed under dimmed light to preserve light sensitive vitamins.

DATA MANAGEMENT

All data were entered using Microsoft

Excel and verified. Statistical analyses were performed using SPSS (version 11; Stata Corporation, USA). Anthropometric Z-scores for weight-for-height (WHZ), length- or height-for-age (LAZ or HAZ), weight-for-age (WAZ) and BMI-for-age (BAZ) were calculated based on the WHO (2006) growth reference data for children <5 years of age. The prevalence of micronutrient deficiencies were assessed according to the recommendations of WHO and expert committee reports. Children with plasma retinol concentrations <20 µg/dl were characterized as being vitamin A deficient and α-tocopherol concentration <500 µg/dl were characterized as vitamin E deficient.

STATISTICAL ANALYSES

Descriptive data were analyzed by mean ± standard deviation (SD). Comparison between two groups was performed by the unpaired Student's t-test and Chi-square test for prevalence (%) while for more than one group one-way ANOVA was used.

RESULTS

The anthropometric data of the study populations is shown in Table 1. There were total 46 pre-school children (29 boys and 17 girls). The group of boys and girls were well-matched with no significant statistical differences among them, including the age, body weight, height, body mass index (BMI) and different z-scores. The prevalence of underweight (WAZ, -2 SD) and thinness (BAZ, -2 SD) was 37% and 13% respectively, while wasting(WHZ, -2 SD) was 17% and stunting (HAZ, -2 SD) was 35% indicative of widespread chronic and acute malnutrition based on the WHO standards (WHO 2006). No significant differences were found in the mean, anthropometric parameters and in prevalence of malnutrition among boys and girls.

The mean biochemical and hematological parameters among children (gender-wise) are presented in Table 2. None of the variables studied showed statistically significant difference between boys and girls.

Table 3 reveals association of vitamin A (retinol) status with hematological and ferritin levels in children. Those children with subclinical level of VAD had significantly lower levels of Hb and HCT values. The RBC count was also found to be lower in VAD children although non-significant while ferritin level was found to be high with borderline of significance.

Table 4 describes the subclinical vitamin E status in association with different hematological values among children. The levels of Hb, HCT, RBC and MON counts were found to be significantly lower among children deficient with vitamin E, while plasma ferritin was significantly higher. However, MCV was found to be lower in children with VED although statistically not significant.

Table 5 describes the distribution of children having combinational VAD and VED status with hematological and ferritin levels. Concentrations of mean Hb, HCT, RBC count were consistently declined significantly in order among children who had free from VAD and VED than who had VAD or VED alone or in dual VAD and VED. On the contrary under similar conditions the GRA and plasma ferritin

levels increased.

DISCUSSION

Globally, almost half of preschool aged children and pregnant women and close to one third of non-pregnant women suffer from anemia which is not only due to iron deficiency but also due to deficiency in other vitamins. Fat-soluble vitamins A and E are important non-enzymatic antioxidants (Allard et al., 1998). Our result indicates that lowering of vitamin A level influences mostly lowering of Hb, HCT while increasing ferritin level as evidenced by many studies supporting a view that hematopoiesis may have been mediated by improved vitamin A nutriture. Concentrations of transferrin receptor and plasma ferritin both declined steadily with increase in vitamin A level while their ratio remained unchanged, suggesting that while total body iron remained unchanged, hepatic iron had been mobilized to support erythropoiesis (Evans, 2005). A study conducted in Guatemala where daily vitamin A fortified sugar was given to children had shown increase in serum retinol and iron while decrease in serum ferritin, suggesting that tissue iron had been mobilized following improved vitamin A status (Mejia and Arroyave, 1982). Vitamin A supplementation significantly increased blood Hb, HCT, RBC, serum iron and transferrin saturation while no effect on total iron binding capacity or ferritin in anemic children (Mejia and Chew, 1988). In Thailand, children given a single 60 mg (200,000 IU) dose of vitamin A led to increase in serum iron, percentage of transferrin saturation, Hb and HCT after two weeks as compared to controls (Alarcon et al., 2004). In a trial conducted in Morocco mild VAD children vitamin A supplementation increased Hb as well as plasma erythropoietin (Zimmermann et al., 2006). In another study conducted on school-aged children in rural Kazakhstan, Hb was significantly correlated with retinol concentrations (Hashizume et al., 2004). Ahmed et al., reported significant correlations between the concentrations of serum retinol, serum iron, Hb, HCT, and MCH in adolescent girls in urban Bangladesh.

Children are more prone to vitamin E deficiency as newborn infants have low circulating concentrations of vitamin E owing to placental impermeability (Wright et al., 1951), and transient lipoprotein deficiency at birth (Desai et al., 1984). In this study, low levels of Hb, RBC and HCT observed in VED children suggesting a possible role of vitamin E in improving certain hematological indicators. It was observed that disturbances in some of the hematological parameters like higher osmotic fragility of RBC and lower MCV, MCH, MCHC occur in G6PD deficient hemolytic anemic patients returned towards normal after supplementation of vitamin E, indicates clearly the role of antioxidant in maintaining red cell membrane integrity and thereby decreases the rate of haemolysis (Nayma et al., 2008). Supplementation with α-tocopherol significantly increased packed cell volume (HCT), Hb concentration and percent foetal Hb in sickle cell patients (Jaja et al., 2005). Vitamin E status can be compromised during anemia as a result of the oxidative stress caused by erythrocyte haemolysis. The regeneration of tocopherol from the tocopheroxyl radical in the RBC membrane is especially relevant to the discussion of oxidative stress causing RBC damage, destruction and ultimately anemia (Attri et al., 2006; Traber and

Kamal-Eldin, 2007), although March et al. demonstrated that hypervitaminosis E induced reticulocytosis, lowered HCT value. Moreover, supplementation of vitamin E is shown to influence leucocyte functions (Prasad, 1980). In vitro addition of vitamin E has been reported to depress mixed lymphocyte cultures (Mann et al, 1969). In our study, the group of children deficient with vitamin E have higher lymphocyte count than group deficient with vitamin A, which is even still higher when children with VAD added to VED. In this study plasma ferritin is found to be high in children with VED. Studies using rat as animal model have shown that, α-tocopherol supplementation affected the liver content of lipid soluble antioxidants, suggesting a concerted antioxidant response at the cellular level to modulate the effect of excess iron availability (Galleano and Puntarulo, 1997). Vitamin E and synthetic antioxidants are shown to protect vitamin E deficient piglets from iron toxicity (Tollerz and Lannek, 1964).

Our study shows that children deficient in both these vitamins have very low level of Hb, RBC, HCT values and elevated serum ferritin revealing dual effect on hematological parameters. Studies have shown that long-term supplementation with the combination of beta-carotene and vitamin A increased serum concentrations of α-tocopherol in human subjects (Thurnham et al., 1988; Goodman et al., 1994), which may in turn reduce lipid peroxidation and further prevent RBC haemolysis more effectively. In an experiment when rats fed with a combination of β-carotene and α-tocopherol, lipid peroxidation caused due to iron overload is greatly reduced (Whittaker et al., 1996). Optimal plasma concentrations of vitamins A and E are essentially required for leukocyte function (Eicher et al., 1994). In our result granulocyte count is found to be significantly high in children with lone VAD or VED or in children with dual (VAD and VED) deficiencies.

CONCLUSION

This study reveals that plasma levels of vitamins A and E greatly influence the Hb, HCT, RBC count as well as iron overload in the body. Both these vitamins affect the hematological parameters independently as well as combined. The protective effect of vitamin E on RBC can be enhanced by vitamin A as deficiency of both results in a severe form of anemic condition as evidenced by low Hb, HCT and RBC count. As substantiated from our data, it seems that the conservation of RBC is more effectively mediated by vitamin E as compared to vitamin A. Precisely why; there is a need to design tools that combine vitamin E along with existing vitamin A prophylaxis programme for effective control of nutritional anemia in children.

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Table 1: Mean (±SD) anthropometric characteristics of preschool children

Parameters	Total (n=46)	Boys (n=29)	Girls (n=17)	'P' value
Age (months)	36.01±18.74	38.29±18.25	32.13±19.48	0.286
Body weight (kg)	11.29±4.17	12.11±4.37	9.76±3.37	0.063
Height (cm)	86.95±17.69	90.08±17.99	81.12±16.08	0.097
Body mass index (BMI kg/m2)	14.36±1.89	14.43±2.26	14.25±0.92	0.756
Weight-for-age z-score	-1.85±1.41	-1.80±1.64	-1.94±0.88	0.751
Height-for-age z-score	-1.81±1.88	-1.75±2.19	-1.91±1.18	0.776
Weight- for-height z-score	-1.18±1.57	-1.25±1.85	-1.06±0.89	0.703
Body-mass index-for-age z-score	-1.09±1.63	-1.12±1.96	-1.06±0.76	0.906
Underweight (WAZ <-2 SD %, n)	36.95 (17)	31.03 (9)	47.05 (8)	0.277
Stunting (HAZ <-2 SD %, n)	34.78 (16)	34.48 (10)	35.29 (6)	0.955
Wasting (WHZ <-2SD %, n)	17.39 (8)	20.68 (6)	11.76 (2)	0.594
Thinness (BAZ <-2SD %, n)	13.04 (6)	17.24 (5)	5.88 (1)	0.269

WAZ=weight-for-age z-scores, WHZ=weight-for-height z-scores, HAZ=height-for-age z-scores, BAZ=BMI-for-age z-scores.

Table 2: Mean (±SD) hematological characteristics of the preschool children

Blood Parameters	Total (n=46)	Boys (n=29)	Girls (n=17)	'P' value
Plasma vitamin A (retinol µg/dl)	34.56±23.00	35.76±26.77	32.52±15.05	0.649
Plasma vitamin E (α-tocopherol µg/dl)	645.3±532.0	576.2±476.1	759.1±611.3	0.264
Haemoglobin (g/dl)	10.12±3.38	10.59±3.19	9.31±3.64	0.217
WBC (thousand/mm3)	9.08±6.06	9.51±6.43	8.118±5.45	0.456
RBC (million/mm3)	4.07±1.38	4.21±1.15	3.823±1.71	0.363
HCT (%)	33.10±11.38	35.20±10.25	29.52±12.60	0.102
MCV (fl)	82.68±10.11	83.58±6.96	81.15±14.09	0.437
MCH (pg)	25.17±3.84	25.41±2.71	24.76±5.32	0.585
GRA (%)	36.16±17.61	36.40±17.49	35.76±18.35	0.906
MON (%)	6.02±4.08	6.76±4.75	4.81±2.30	0.119
LYM (%)	57.14±16.09	55.88±15.28	59.22±17.62	0.502
Plasma ferritin (ng/ml)	192.4±241.3	192.3±247.1	192.5±239.1	0.997

Table 3: Association of vitamin A (retinol) status with hematological and ferritin levels in children

Hematological and Iron Indicators	Vitamin A (retinol) status		'P' value
	Normal (retinol ≥20µg/dl)	Vitamin A deficient (retinol <20 µg/dl)	
N (%)	30 (65.2)	16 (34.8)	-
Haemoglobin (g/dl)	10.99±2.79	8.47±3.86	0.014*
WBC (thousand/mm3)	8.56±6.64	9.82±4.88	0.507
RBC (million/mm3)	4.32±1.16	3.60±1.66	0.095
HCT (%)	35.58±9.80	28.45±12.94	0.042*
MCV(fl)	84.41±10.29	79.45±9.19	0.113
MCH(pg)	25.54±4.03	24.48±3.47	0.378
GRA (%)	33.37±18.55	41.73±14.56	0.125
MON (%)	6.410±4.20	5.25±3.87	0.363
LYM (%)	59.19±16.87	53.03±14.02	0.219
Plasma Ferritin (ng/ml)	144.1±219.7	279.9±261.0	0.068

*Significance $p < 0.05$

Table 4: Association of vitamin E (α-tocopherol) status with hematological and ferritin levels in children

Hematological and Iron Indicators	Subjects α-tocopherol level (≥500µg/dl)	Subjects α-tocopherol level (<500µg/dl)	'P' value
N (%)	26 (56.5)	20 (43.5)	-
Haemoglobin (g/dl)	11.82±2.34	7.90±3.30	0.0001**
WBC (thousand/mm3)	7.85±6.70	10.48±4.87	0.147
RBC (million/mm3)	4.67±1.10	3.28±1.34	0.0004**
HCT (%)	38.38±8.04	26.25±11.58	0.0001**
MCV(fl)	84.95±9.84	79.73±9.91	0.082
MCH(pg)	25.85±4.27	24.29±3.08	0.174
GRA (%)	32.61±17.76	40.60±16.81	0.128
MON (%)	7.204±3.94	4.55±3.86	0.027*
LYM (%)	60.04±17.88	53.51±13.07	0.176
Plasma Ferritin (ng/ml)	103.3±163.1	303.7±278.9	0.004**

*Significance $p < 0.05$
**Significance $p < 0.01$

Table 5: Combinational status of vitamin A and vitamin E with hematological and ferritin levels in children

Hematological and Iron Indicators	Children with no VAD and VED	Vitamin A (retinol <20 µg s/dl) deficiency alone	Vitamin E (α-tocopherol <500 µg/dl) deficiency alone	Vitamin A and Vitamin E deficiencies combined	F value	'P' value
N (%)	8 (17.4)	18 (39.1)	12 (26.1)	8 (17.4)	-	-
Haemoglobin (g/dl)	12.07±2.39	11.24±2.26	9.37±2.631	5.70±3.06	12.30	0.0001**
WBC (thousand/mm3)	7.27±7.55	9.18±4.38	10.50±4.62	10.46±5.57	0.886	0.456
RBC (million/mm3)	4.62±1.22	4.79±0.79	3.86±0.93	2.41±1.43	8.329	0.0002**
HCT (%)	38.49±9.34	38.13±4.31	31.23±9.15	18.78±11.24	9.856	0.0001**
MCV(fl)	86.76±9.06	80.89±10.94	80.87±11.40	78.01±7.53	1.859	0.151
MCH(pg)	26.37±4.22	24.70±4.42	24.30±3.53	24.26±2.49	0.979	0.411
GRA (%)	27.44±16.01	45.90±15.81	42.28±19.17	38.08±13.31	3.201	0.033*
MON (%)	7.11±3.77	7.443±4.65	5.36±4.74	3.32±1.54	2.152	0.108
LYM (%)	65.24±16.96	46.66±13.17	50.12±12.50	58.60±12.99	4.145	0.011*
Plasma Ferritin (ng/ml)	91.41±186.2	128.6±104.3	218.7±249.1	431.1±287.7	4.939	0.005**

*Significance $p < 0.05$
**Significance $p < 0.01$