__Original paper__

EFFECTS OF ANTIOXIDANTS ON ADVANCEMENT OF MENTAL AGE AND SCHOOL PERFORMANCES OFPRESCHOOL CHILDREN

S M Hossain[1] A S M M Rahman[2], MA Malek[3], M A H Bhuyari[4]. A Hai[5], G M Chowdhury[6]

Abstract

This study was designed to determine the effects of antioxidants on mental age and school performances of underprivileged preschoolers in two Government Primary Schools of Dhaka city. Ice cream fortified with beta-carotene, vitamin E, vitamin C and zinc at recommended doses and time duration was given to the study children (n=117). Equal quantity of plain ice cream was fed to the control children (n104) for the same duration. In study group and in controls the aseline mean serum level of p-carotene, vitamin E, were similar. At the end of intervention the study group bad serum level of 3-carotene as 22 mcg/dl, vitamin E as 938 mcg/dl, vitamin C as 0.96 mgldl and zinc as 1.11 mgldl. The same in control group after intervention came to be as B-carotene-4.8O mcg /dl, vitamin E-326 mcg Id!, vitamin C-0.15 mg/dl and zinc- 0.53 mg/dl. Mental age assessment highlighted that the study children bad mental age before intervention base line as; normal and advanced-21.4% and retarded- 78.6% which after intervention came to be normal and advanced-88%, retaçded-12% with p-value = <0.0001. But in control children it was found to be before intervention as normal and advanced-23.1% and retarded-76.9% which after intervention came to be as normal and advanced-24% and retarded-76%. Changes in the school performances of the study children in respect of mean total marks obtained were before intervention-311, after intervention-428 with paired t-test (0.000). But control children showed it to boas before intervention-328, after intervention-331. It can be concluded that food fortification can be an important tool to improve the nutritional and mental state of underprivileged school children.

Key words: Antioxidants, mental age/IQ, school performances, intervention.

Introduction:

Oxidative stress is the product of generaton of Reactive Oxygen Species (ROS). A wide variety of free radicals and other ROS can be formed in the human body and in food system. These free radicals can attack lipids in cell membrane, destroy cellular enzyme, and even damage the genetic material. The damaged DNA is replicated in new cells causing slow deterioration resulting in signs of aging and the development of heart disease, cancer and cataracts etc. compromising the life span. Oxidative stress produces toxic consequences at sub-cellular level. Malnutrition is a serious problem in Bangladesh and it makes children more vulnerable to all kinds of infections. Out of total children deaths in Bangladesh in a year 65% are directly or indirectly attributable to malnutrition[1].

Antioxidant defenses protect the body against the oxidative damage2. Vitamin E is a natural antioxidant and acts by donating hydrogen from hydroxyl group. It has its major biological role in protecting polyunsaturated fats and other components of the cell membrane. It is a strong free radical scavenger and in addition contributes to maintenance of cell integrity, anti-inflammatory effects, DNA synthesis and stimulation of immune response. Vitamin E also plays an important role in T-cell differentiation in thymus. Deficiency of vitamin E may cause shorter life span of erythrocvte. Beta-carotene is also a very strong antioxidant with its free radical scavenging property. Carotenoids are known to deactivate free radicals and excited oxygen, both of which are implicated in a multitude of degenerative diseases3. Carotenoids prevent lipid peroxidation by providing electrons to quench singlet oxygen. Beta- carotene is capable to protect chemically induced toxicity in vivo as well as in vitro. Besides being a potential source of vitamin A for infants in breast milk, B-carotene may also confer long-term protection against chronic disease such as cancer and enhance the immunoprotective effect of human milk. It is known as pro-vitamin A of which about 30% is converted into vitamin A. So, it helps to bring the serum retinol level to normalcy and maintains.

1. Lt Col Sarder Mahmud Hossain, MBBS, DHM, Ph.D.
2. Maj Gen (Retd) ASM Matiur Rahman, MBBS, MCPS, D. Bact, MSc. FCPS (BD). FCPS (Pak), FICS, FRCP, FAS.
3. Prof. Md. Abdul Malek. Ph.D
4. Prof. Md. Aminul Haque Bhuyan, Ph.D
5. Col Abdul Hai, MBBS MCPS. FCPS
6. Lt Col Golam Mohiuddin Chowdhury, BDS, FCPS

Integrity and normal functions of glandular and epithelial tissues, increases immunity of the child and fights against infection[4]. Vitamin C is another effective scavenger of free radicals and its plasma concentration is invereIv related to mortality from all causes[5]. Ascorbate s an essential enzyme cofactor but often regarded as an important antioxidant in vivo, protectmg agairt cancer by scavenging DNA damaging reactive oxygen species. It is water-soluble, quite potent. provides electrons for the regeneration of vitamin E. thus potentiating the amioxidant action. Zinc is also an important anticxidant. It scaenges free radicals, thus preventing lipid peroxidation and cell membrane damage. It is present in more than 70 metalloenzymes which carry various metabolic activities[6]. Most of them are involved in DNA and protein synthesis. Zinc deficiency results in poor growth, poor appetite in infants and children and also weight loss[7]. Dramatic weight gain was observed in children with zinc supplementation. It favors lean tissue formation and heals infectious and non- infectious skin lesions. Anorexia, diarrhea, stunting, wasting etc. are relieved and improved cell mediated immune (CMI) response is observed with zinc supplementation. Mild zinc deficiency can cause abnormalities of immune functions. Zinc contributes to membrane stabilization. When antioxidants ai' given together, vitamin E and C show strong synergistic action. Vitamin F and beta- carotene also have synergistic action but tamin C and 13- carotene have not yet shown any action as such5. Zinc has not been combined before along with these antioxidants hut it might have played a good synergism with 'itamin E and C as well as beta-carotene. Methods and Materials Study Design. The study was designed and conducted among preschool children in some selected Government Primary Schools of Dhaka city. The whole class either play group; nursery or kindergarten was taken either as experimental or control group. Beta-carotene, vitamin E. vitamin C and zinc were added to 100gm of ice cream in recommended doses like 2mg, 6mg, 30mg and 10mg respectively. It was for daily consumption, 5 days a week for a period of six months by the experimental group. The control group was served with same ice cream without antioxidants. Any individual making himself/herself absent from school for 15% of the total classes was considered as a dropout case. Also the student having any chronic illness was excluded from the study. Before the start of the intervention the whole study population was dewormed by administering antihelminthic drugs.

Study instrument:

A pretested, modified and corrected questionnaire was used to collect the required information.

Biochemical Assessment:
The blood sample was collected and preserved in sterile tet tubes and kept its upper end scaled to avoid possible contamination. Each time the collected sample was transported to the laboratory within an hour. There the samples were centrifuged at 3000 rpm to isolate the serum Serum level of vitamin E. vitamin C, beta-carotene an' zinc were measured at 0 and 180 days of the study period to find out the changes due to intervention. The serum level of beta-carotene and vitamin E was measured by HPLC method. Plasma ascorbic acid level was measured by dinitro phenyl hydrazine method and zinc was assessed by Association of Official Analytical Chemist (AOAC) methods of analysis. These tests were done in the Department of Biochemistry, ICDDR, Dhaka, and Department of Biochemistry, Institute of Nutrition and Food Science (INFS), University of Dhaka.

Seguin Form Board Test (SFBT):
This method was followed to test the mental age of the children. It is a traditional method which employs some toy like torn, and asks the subject to set it in order. On the basis of time required to complete the task mental age is recorded. Statistical analysis: The data were computed and analyzed by standard statistical method using SPSS software package.

Results:
Mean serum level of all antioxidants under study was assessed on 0 and 180 days of intervention. Table-I revealed that serum level of 13-carotene in experimental group before and after intervention as 5.11±2.41 mcg/lOOml and 22.64±3.72 mcg/lOOml respectively (paired t-test = 0.000) whereas the control children had the same before and after intervention as 5.29±2.46 mcg/lOOml and 4.80±2.26 mcg/lOOml respectively.

Table I: Changes of mean beta-carotene in serum (mcg/lOOml) of study children before and after intervention

Serum Beta – carotene	**Experimental Mean±SD; (n=117)**	**Control Mean±SD; (n=104)**
Before intervention	5.11±2.41	5.29±2.46
After intervention	22.64±3.72	4.80±2.26
Difference	17.53±3.19	(-)0.49±1.23
Paired t-test	0.000	0.000

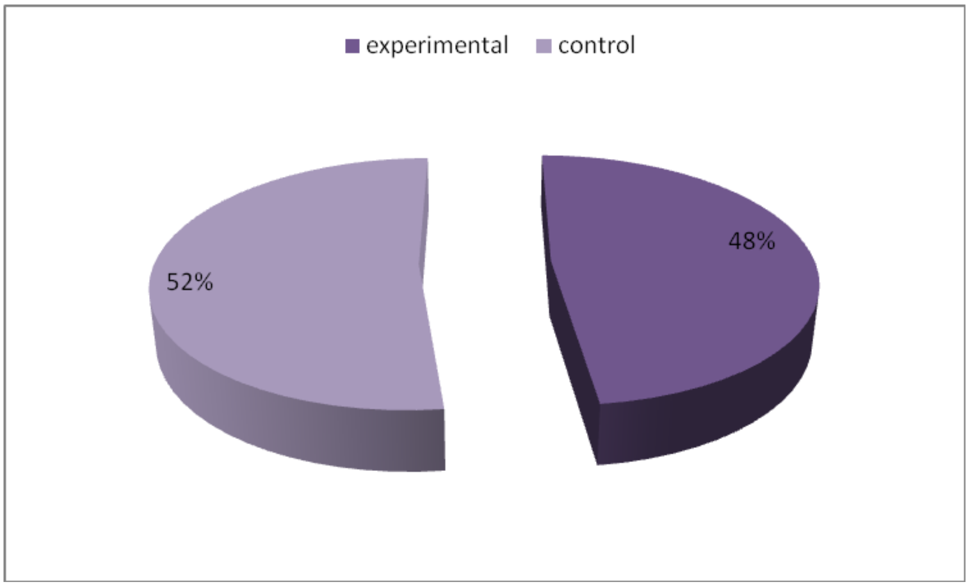

Fig 1: Percent Distribution of Mental age study Children in the Experimental and Control Children before Intervention

Table-II showed that the mean serum level of vitamin E of experimental children before and after intervention as 307.69±157.44 mcg/l00 ml and 938.27±163.64 mcg/l00ml respectively with paired t-test 0.000. On the other hand the same in control children before and after intervention was found to be 330.52±73.93 mcg/100ml and 326.53±72.54 mcg/l 00ml respectively.

In the same way table-III revealed the serum level of vitamin C in experimental children before and after intervention as 0.16±0.07 mg/l00ml and 0.96±0.28 mg/l00ml respectively with paired t-tcst=0.000. But the same in control children before and after intervention was found to be as 0.14±0.05 mg/l00ml and 0.15±0.06

Table II: Changes of Mean Serum vitamin E (mcg/l00ml) in Study Children before and after Intervention

Serum Beta – carotene	Experimental Mean±SD; (n=117)	Control Mean±SD; (n=104)
Before intervention	0.16±0.07	0.29±0.06
After intervention	2.964±3.282	4.80±2.06
Difference	17.803±3.279	(-)0.0490.043
Paired t-test	0.000	0.000

Fig 2: Percent Distribution of Mental age study Children in the Experimental and Control Children before Intervention

mg/ 100ml respectively.

It was highlighted in Table-IV that the mean serum level of zinc in the experimental children before and after intervention was 0.52±0.llmg/l00ml and 1.11±0.14 mg/100ml respectively with paired t-test 0.000. On the contrary the same in control children before and after intervention was found to be 0.52±0.09 mg/l00ml and 0.53±0.09 mg/100ml respectively.
Figure-I depicted the percent distribution of mental age of experimental children before intervention as normal & advanced- 25(21.4%), retarded- 92(78.6%). The control children showed it to be as normal & advanced-

Table III: Changes of Mean Serum Vitamin C Level (mg/I00ml) in Study Children before and after Intervention

Fig 3: Average Changes in School Performances of Study Children before and after Intervention

24(23.1%), retarded- 80(76.9 %.)

But figure-2 showed that the experimental group had mental age after intervention as nornal & advanced 103(88%), retarded-14(12%) with p-value <.0001. But the control children showed it to be normal & advanced 25(24%), retarded-79(76%) with p-value = >0.7.

Figure-3 highlighted the state of school performances in respect of mean total marks obtained by the study children, The figure showed that experimental and control children got the mean total marks before intervention as 311.55 65.55 and 328.38 92.78 respectively. But after intervention

Serum Beta – carotene	**Experimental Mean±SD; (n=117)**	**Control Mean±SD; (n=104)**
Before intervention	0.52±0.11	0.52±0.09
After intervention	1.11±0.14	0.53±0.09
Difference	0.59±0.14	0.01±0.06
Paired t-test	0.000	0.333
Serum Beta – carotene	**Experimental Mean±SD; (n=117)**	**Control Mean±SD; (n=104)**
Before intervention	0.52±0.11	0.52±0.09
After intervention	1.11±0.14	0.53±0.09
Difference	0.59±0.14	0.01±0.06
Paired t-test	0.000	0.333
Serum Beta – carotene	**Experimental Mean±SD; (n=117)**	**Control Mean±SD; (n=104)**
Before intervention	0.52±0.11	0.52±0.09
After intervention	1.11±0.14	0.53±0.09
Difference	0.59±0.14	0.01±0.06
Paired t-test	0.000	0.333

Table IV: Average Changes of Serum Zinc Level (mg /l00mI) in Study Children before and after Intervention

Mean total marks obtained by experimental children was 427.99 81.66 ith paired t-test =0.000 whereas the same obtained by control children was 331.11 88.69 but without any significance.

Discussion:

The normal serum level of beta-carotene; vitamin E, vitamin C and Zinc are 50-250 .ig/dl, 500-1500 .tgJd1, 0.4- 1.0 mg/d1[9] and 0. LO2mg/dl[10] respectively. The normal level of antioxidants in serum clearly indicated that the children under study had subnormal level of all the antioxidants except zinc, it had been concluded that these antioxidants were absolutely essential for normal growth and development, for development of immunity to lead a healthy life.

But why the changes in mental age (IQ) as well as it school performances in experimental children so happened? It is known that majority of the intellectual disabilities in adults reflect insults to the brain that occurred early in life[11] Long-term effects of malnutrition was identified by Dr. Janina Galler and her colleagues in a cohort study here she found that in spite of excellent rehabilitation and follow up provided to the group of previously malnourished infants, 60-70% demonstrated symptoms of attention deficit in the class room, poor memones and easy distractibility, as well as poor overall school performances'[12].

In another long-term study of Costa Rican infants the problems in their behavior and development were recorded Even after ten years of the treatment for iron deficiency. It was observed in a recent study in rats that pcrinatal iron deficiency reduces neuronal metabolic actiity, especially targeting those areas of the brain which are involved in memory processing[13]'. Lozoff noted the in reasing evidence that maternal iron deficiency may cause injury to the Ital brain. Very often micronutrieni deficienccs are associated with generalized under nutrition, in this way young children may experience multiple nsults to their developing braijis'[14]. Serum level of zinc also inversely affects mental age in the children.

One more important point has been also ioted as a reason for poor mental age of the study children related to retarded growth in the childhood. There are consislent evidences for associations between linear growth reta'dation and poor cognitive outcome. Longitudinal studies indicate that deficits to cognitive ability remain in later childhood[15]' and adult life. Early height has also been shown to predict intellectual ability in young adults (average age 20 years) in Guatemala'[16]. In a cohort of stunted and non-stunted Jamaican children from 9-24 months, stunted children's developmental levels were significantly below those of non-stunted children.

Behavioural changes in stunted children may contribute to their poor cognitive development. Stunted children have been shown to be significantly more apathetic, less happy, and fussier and to explore their environments less than non-stunted children. These behavioral differences predicted late development'[17].

As the reasons of poor mental age in children are looked into carefully, it was seen that almost all causes were present in the children under study in our situation. The overall trend of child malnutrition in Bangladesh is one of the highest in the world. Overall stunting is 45%, underweight is 48% and wasting is 10%. (So: WB, 2002a.) Deficiency of iron, iodine, vitamin A etc. is also a big public health problem. Morbidity and mortality due to Pneumonia, Diarrhea and other nfectious and communicable disease are significant n this country. Micronutrient deficiency was present in the study population as evaluated by biochemical asessment in this study. Though deworming was done in both the study groups, micronutrient deficiency was corrected only in experimental children due to intake of fortified ice cream. All the micronutrients were unique antioxidants. Trace element like zinc was also supplernentee as antioxidant.

So. in addition to correcting the micronutrient deficiency, the supplementation program improved vitamin A status as well as absorption through gut. All antioxidants played a vital role to combat chronic as well as acute illnesses thus aerting the insult to the brain. Also the experimental children in this study got corrected their stunting and had significantly improved linear growth which has not been elaborated here. On the other hand it did not happen with the control children. As such, it is assumed that antioxidants individually, combinedly as well as synergistically contributed to improving the mental age of the study children significantly. The insignificant improvement of mental age as experienced in the control children could he due to effective deworming as a part of the study and improvement of nutritional status through 100gm ice cream intake every school day.

School performances of the study children were evaluated with care before and after intervention. It was noticeable that a baseline mean total mark in experimental children was less than control children. After intervention the difference in mean total marks in experimental children was 116.44+56.22 whereas in control children it was only (2.72+24.9)). In a good reasoning it could be brought forward that helminthic infestations are very common in our country. It is associated with learning problems. Heavy worni infestations have negative effects on memory and the executive functioning[18]. Few other studies have reported about impaired attention, searching ability and reaction time. There is also evidence of poor school performance by school children with parasitic infestations18. It can be further told that all experimental children of the study group comparatively attained a good health, overcame the common morbidities and attained a good immune status. Supplementation with antioxidants reduced the adverse effects of oxidative stress, further improved conditions for a good health. These factors increased the working hours of the students in the school, minimized the loss of working hours due to reduced illness as well as getting a better concentration for their studies. This finding is supported by the school feeding program (SFP) in Bangladesh in 2002 in collaboration with World Food Program (WFP). The biscuits were enriched with protein, iron and vitamin A. The feeding program improved not only the Body Mass Index (BMI) of the participating children by 0.62 points but also school performances like increasing test score by 15.7% and doing well specially in mathematics’9. Improved school performances also support the improvement of mental age of the experimental children.

Conclusion:

Hunger and undernutrition along vith antioxidant micronutrients deficiency pre au amons a vast mass of population in this country. These antioxidants are essential for normal development particularly among young children. Steps may be taken to fortify such foods with antioxidant micronutrients accessible to all. It will bring out a healthy, intelligent and prosperous nation in the days to come

References:

1.Baqui AH, Black R, Arefin SE et al Causes of childhood deaths in Bangladesh; Results of a nationwide aucopsy study Bulletin of world Health Organization; 1998: 76:161-17l

2. Halliwell B. Murcia MA. Chirico S. Aroma oi Free radicals and antioxidants in food and in vivo: What they do and how they work cit Rev Food .Sci Nutr Jan 1995:35(1-2):7-20

3. Ames BN Dietary carcinogens and anticar:r z-is and degeneratisc diseasesi. cnce. ‘3. 221 125

4. K. Park. Text Book 01 Pres enti\ e and Soc:. nediine. ‘\:c ii cdition,2000: pp-409.

5 Khaw K F, Bingham S. Welch A, Luhen R, Warelutm N. Gakes S. l)a N . Relation between plasma ascorbic acid and mortality in men and women in EPIC—Norfolk prospectit c study: a rspective population study. European Prospectis c Insestigation into •anccr and Nutrition. Lancct Mar 2001: 357(9257): 657-63.

(,. Schofield C, Ashworth A . Wh’, hase mortainy rates for ses etc malnutrition remained so high? Bull WI-tO; 1996, 74: 223-29.

7. Hambidge KM, Hambidge C, Jacob M. Baum JD . Low levels of Zinc in hair, anorexia, poor growth and hypogeusia in children. Pediatr Res:
1Q72; 6: 868-74.

S. Niki F. Noguehi N, Tsuchihashi H, Gotoh N. InteTaction among vitamin I.. itainiti 1. and beta-carotene. Am J (un Nutr Dec:1995; 62(6 Suppl). 322S-l3265.
0. Gerald F. Loud s. Jr. The vitamins, second edition;1998: pp-I 50, 208, 2 4, 217 and 251
10. Eric J. Undersvood, Trace Elements in Human and animal nutrition. Fourth edition: Academic press New York: 1997; pp-20l.
II. Karen ()lness. The global burden-Conditions that adversely and significantly affect learning. Annales Nestle: 2001; 59: 89-99.
12. Galler JR, Ramsey FL, Solimano G; The influence of early malnutrition on subsequent behavioural development. 111. Learning disabilities as a sequel to malnutrition. Pcdiatr Res: 1984; 18: 309-313.
13. Deungria NI, Rao R, Wobken JD. et al. Perinatal iron deficiency decreases cytochrorne c oxidase (CytOx) activity in selected regions of neonatal rat brain. Pediatr Res: 2000 48: 169-76.
Karen Oness. The global burden-Conditions that adversely and 5ienitkantls aftet learning. Annales Nestle; 2001; 59: 89-99.
5. \lendez MA. Adair LS. Severity and timing of stuntmg in the first t’; n7ccz performance on cognitise :ests in late childhood.’? \:.:r 1_.,:Z0 555-62
It \lartji.i R. Risera J. Kapiossitz H. Pollitte E. Long-term COflseqUCflCe of u: os’. :h retarcanon darirg early 1991.
7 Meeks Gardner JM. Grantham-MGregor SM. Himes J. Chang S. Behaviour and development of stutied and non-stunted Jamaican children .J(hild Psychol Psychiatry: l99: 40: 819-27.
IS. Nokes (. Gra:itham-McGregor SM. Sawyer AW et at. Moderate to heir, y infections of Trichuris trichiura affectixe cognitive functions in Jamaican school children. Parasitology: 1Q92; 104: 539-47.
19. Akhtcr U Ahnied. ‘impact of Feeding Children in School: Evidence from Bangladesh” International Food Policy Research Institute, Washington, DC: 2004.