Role of Antioxidants for Optimum Growth among Preschool Children

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Abstract— A double blind interventional study was carried out in three Government Primary Schools to see the changes in growth which revealed the baseline serum level of beta-carotene, vitamin E, vitamin C and zinc for interventional (n=117) group as 5.11 mcg, 307.69 mcg, 0.16 mg, 0.52 mg and control group (n=104) as 5.29 mcg, 330.52 mcg, 0.14 mg and 0.52 mg respectively. The serum level of the same antioxidants after 6 months of feeding a cup of 100ml ice cream, five days a week, fortified with antioxidants in recommended doses in interventional group showed to be 22.64 mcg, 938.27 mcg, 0.96 mg and 1.11 mg (Paired t-test=0.000) whereas the control group having ice cream without antioxidants had the serum level as 4.80 mcg, 326.50 mcg, 0.15 mg and 0.53 mg respectively. Impact on growth in interventional group was observed as weight (kg)-(base line-16.24, after intervention-21.36), Height (cm)-(base line-107.24, after intervention-113.91), TSF (mm)-(base line-7.11, after intervention-8.44) and MUAC (cm)-(base line-15.25, after intervention-17.66). But in control group the impact on growth was weight (kg)-(base line-16.99, after intervention-18.63), height (cm)-(baseline-108.11, after intervention-109.98), TSF (mm)-(base line-6.99, after intervention-7.23) and MUAC (cm)-(base line-15.86, after intervention-16.10) clearly indicating a higher improvement in growth of the interventional study children. Fortification of staple food stuffs with essential antioxidants is recommended to overcome the deficiency state.

Index Terms— TSF, MUAC, Serum level, Baseline, Intervention, antioxidants

1 INTRODUCTION

OXIDATIVE stress is stated as an imbalance in the rate at which the intracellular content of reactive oxygen species (free radicals) increases relative to the cell to eliminate free radicals [1]. Activated oxygen and agents that generate oxygen free radicals, such as ionizing radiation, induce numerous lesions in DNA that cause deletions, mutations and other lethal genetic effects. These free radicals can also attack lipids in cell membrane, destroy cellular enzyme, and even damage the genetic material – deoxyribonucleic acid. If the injury to DNA by free radicals is not totally repaired, the damaged DNA is replicated in new cells and slow deterioration occurs. It is this gradual deterioration that is likely to be responsible for the familiar signs of aging and the development of heart disease, cancer and cataracts, consequently compromising the life span [2]. Oxidative stress is also believed to contribute to numerous pathological conditions like atherosclerosis, obstructive lung disease, aging, and fatigue of skeletal muscles including the diaphragm [3]. Malnutrition makes the people of all ages particularly children more vulnerable to oxidative damage. In Bangladesh, malnutrition is a major child health problem and about 56% of Bangladeshi children having age under five suffers from moderate to severe malnutrition and 21% of them are severely underweight [4]. About 13 million children die every year in the world, 95% of them in developing countries where pneumonia (infection) is one of the leading causes, accounting for about four million deaths [5]. About 55% of the total children who die every year in the world are directly or indirectly due to malnutrition. In Bangladesh the problem is worse. Out of total death of children in a year, 65% are directly or indirectly attributable to malnutrition [6].

Antioxidants neutralize the destructive actions of free radicals generated as a result of normal oxidative process in human body. The antioxidants, both primary and secondary, interfere at different stages in the oxidation process but they have a mutually reinforcing effect on each other. When they are used together, the overall inhibiting effects get generally greater than the sum of the individual effects from each antioxidant. β-carotene is a natural antioxidant and an effective quencher of singlet oxygen [7-8]. Carotenoids are known to deactivate free radicals and excited oxygen.

Vitamin E is also a natural antioxidant; ascorbic acid and tocopherol together act synergistically. Vitamin E has its major biological role in protecting polyunsaturated fats and other components of the cell membrane from oxidation by free radials, which is believed to be due to its antioxidant activity [2]. In addition vitamin E plays important roles in other biological processes which include maintenance of cell integrity, anti-inflammatory effects,

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DNA synthesis and stimulation of immune response.

Vitamin C is also an effective scavenger of free radicals, have synergistic action along with vitamin E and its plasma concentration is inversely related to mortality from all causes, and from CVD, and IHD in men and women [9]. Vitamin C, a water-soluble antioxidant is quite potent and can provide electrons for the regeneration of vitamin E thus potentiating the antioxidants' action in vivo [10].

Zinc too plays an important role as an antioxidant. It is engaged in scavenging free radicals, thus preventing lipid peroxidation and cell membrane damage. It is present in more than 70 important metalloenzymes, which carry various metabolic activities [11]. Most of the enzymes involved in DNA and protein synthesis are zinc metalloenzymes. Anorexia and poor growth are prominent features of zinc deficiency in young animals and are very commonly encountered in case of our children [12-14]. It has been concluded that zinc supplementation increases the rate of weight gain and reduces the energy cost of tissue deposition. It is also mentionable that zinc therapy heals infectious and non-infectious skin lesions [15-16]. Marginal zinc deficiencies in young animals do cause growth retardation and anorexia. This study was undertaken to evaluate the nutritional level of the study population and its overall impact on health status.

2 Study Design

The present work is a double-blind interventional study designed to assess the effects of antioxidants on the growth of children of age 4-6 years in three Government Primary Schools of Dhaka city. Both interventional and control children were matched in respect of socio-economic and demographic parameters. A cup with 100gm of ice cream was fortified with antioxidants such as carotenoids, vitamin E, vitamin C and zinc for daily consumption for the interventional group in the doses of 2000 microgram, 6 mg, 30 mg and 10 mg respectively. The control group was provided with the same ice cream without fortification. Before the start of the intervention the whole study population (interventional and control group) was dewormed.

2.1 Selection Criteria

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.

2.2 Data Collection Instrument

A pre-tested, modified and corrected questionnaire was used to collect the required information.

2.3 Growth Assessment

Baseline and fortnightly information on weight, height, mid-upper arm circumference (MUAC) and triceps skin-fold (TSF) thickness of the study population were collected till the end of the intervention.

2.4 Biochemical Assessment

Biochemical examinations e.g. serum level of vitamin E, vitamin C, carotenoids and zinc were measured at the start and also after 180 days of the study period. Serum level of vitamin E was measured simultaneously by a modification of the method of Bieri et al. (1979) using high-pressure liquid chromatography (HPLC). Plasma ascorbic acid was measured by dinitro phenyl hydrazine method. The serum level of carotenoids was measured by high-pressure liquid chromatography. Serum level of zinc was done by following the Association of Analytical Communities (AOAC) official methods of analysis.

2.5 Statistical Analysis

The data were computed and analyzed by standard statistical method using SPSS software package.

3 Results

Table-1 Shows clearly the mean serum level of β-carotene (mcg/100ml) in interventional group before and after as (5.11±2.41 & 22.64±3.72) and the same in control group before and after intervention as (5.29±2.46 & 4.80±1.23) respectively with a Paired t-test = 0.000.

Table-2 shows the mean serum level of vitamin E (mcg/100ml) in same study population of interventional group before and after intervention to be as (307.69±157.44 & 938.27±163.64) and in control group before and after intervention as (330.52±73.93 & 326.53±72.52) respectively.

TABLE 1

Distribution of respondents by Serum Beta-carotene Level (mcg/100ml)

Serum Beta-carotene	**Interventional** **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

The difference of serum level of vitamin E at the end of intervention was highly significant as shown by Paired t-test= 0.000.

TABLE 2
Distribution of respondents by Serum Vitamin E Level (mcg/100ml)

Vitamin E level in serum	Interventional Mean ± SD; (n=117)	Control Mean ± SD; (n=104)
Before intervention	307.69 ± 157.44	330.52 ± 73.93
After intervention	938.27 ± 163.64	326.53 ± 72.54
Difference	630.57 ± 174.72	*(-)3.99 ± 27.82
Paired t-test	0.000	0.146

*(-) means decreased

Table-3 highlights on the mean serum level of vitamin C (mg/100ml) in interventional group and control group before and after the procedure as (0.16±0.07 & 0.96±0.28) whereas the same in control group is found to be as (0.14±0.05 & 0.15±0.06) respectively. The Paired t-test=0.000 in interventional study population shows the change in serum level to be highly significant.

TABLE 3
Distribution of respondents by Serum Vitamin 'C' Level (mg/100ml)

Vitamin C level in serum	Experimental Mean ± SD; (n=117) mg/100ml	Non-experimental Mean ± SD; (n=104) mg/100ml
Before intervention	0.16 ± 0.07	0.14 ± 0.05
After intervention	0.96 ± 0.28	0.15 ± 0.06
Difference	0.80 ± 0.27	0.01 ± 0.04
Paired t-test	0.000	-

As of the previous cases Table-4 revels the serum level of zinc of both interventional and control groups, the values being (0.52±0.11 & 1.11±0.14), (0.52±0.09 & 0.53±0.09) respectively after 6 months (180days) of successful intervention. This change of serum zinc was statistically significant too (Paired t-test=0.000).

TABLE 4
Distribution of respondents by Serum Zinc Level (mg/100 ml)

Zinc level in serum	Interventional Mean ± SD; (n=117) mg/100ml	Control Mean ± SD; (n=104) mg/100ml
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 5
Distribution of respondents by Weight and Height Gain

Time	Mean Weight (kg) SD			Mean Height (cm) SD		
Before and six months after intervention	**Interventional n=117**	**Control n=104**	**Total n=221**	**Interventional n=117**	**Control n=104**	**Total n=221**
Before	16.24 ± 1.89	16.99± 1.32	16.59 ±1.68	107.24±4.01	108.11± 3.04	107.65± 3.60
After	21.36± 1.92	18.63± 1.29	20.08 ± 2.14	113.91± 3.96	109.98± 3.24	112.06± 4.13
Difference	5.11±0.75	1.64±0.52	-	6.76±2.06	1.87±0.65	-
Paired t-test	0.00	0.00	-	0.00	0.00	-

TABLE 6
Distribution of respondents by Skin-fold Thickness and MUAC

Month	Mean Triceps Skin-fold Thickness (mm) ±SD			Mean MUAC (cm) ± SD		
	Interventional n=117	**Control n=104**	**Total n=221**	**Interventional n=117**	**Control n=104**	**Total n=221**
May 2002	7.11± 1.73	6.99± 1.32	7.06± 1.50	15.25± 1.16	15.86± 0.90	15.54± 1.09
Oct 2002	8.44± 1.69	7.23± 1.18	7.87± 1.59	17.66± 1.16	16.10± 0.90	17.08± 1.20
Difference	1.32 ± 0.62	0.23 ± 0.09	-	2.41 ± 0.60	0.58 ± 0.22	-
Paired t-test	0.000	0.000	-	0.000	0.000	-

Table-5 shows the mean weight gain (kg) in interventional and control children before and after the end of intervention; the result being (16.24± 1.89 and 21.36±1.92), 16.99±1.32 and 18.63±1.29) respectively. In case of height (cm) the same was found to be as (107.24± 4.01 and 113.91± 3.96) and (108.11± 3.04 and 109.98± 3.24) respectively. Paired t-test=0.000 establishes that changes in both the cases were statistically significant.

Table-6 shows similar changes in case of skin-fold thickness and MUAC before and after the intervention in both interventional and control children. Changes in mean skin-fold thickness (mm) was found to be (7.11±1.73 and 8.44±1.69), (6.99± 7.23 and 7.23± 1.18) respectively. And in case of MUAC it was found to be (15.25± 1.16 and 17.66± 1.16), 15.86± 0.90 and 16.10± 0.90) respectively. Here again the outcome of intervention was found to be significant statistically.

4 DISCUSSION

In this study an attempt has been made to find out the combined effects of antioxidants like beta-carotene, vitamin E, vitamin C and zinc in respect of growth of some school children of age group 4-6 years. The study population was 221; of them 117 belonged to interventional and the rest belonged to control group. The study population of both the groups was well matched in respect of education, occupation, living conditions, source of drinking water, health consciousness, sanitation, life-style and monthly income as well as in terms of demographic pattern like family size, pregnancy, state of lactation and birth order.

Significant differences could be observed in the serum level of antioxidants in interventional group and it was raised to normalcy. But changes in the control group were not statistically significant. All such increase of antioxidants in the serum level of interventional group was attributed to intervention program. But same was not true in the control group.

The growth of the study children was assessed by anthropometric measurements. Weight, height, triceps skinfold thickness (TSF) and mid-upper arm circumference (MUAC) were recorded before and after intervention. The mean baseline weight in kg of the interventional and control children was found to be (16.24±1.89) and (16.99±1.32) respectively. After intervention the weight was found to be (21.36±1.92) and (18.63±1.29) respectively. The weight gain in interventional group was significant as observed by Paired t-test (0.000) but it did not happen in control children.

Throughout the study normal diet and growth in the children were not interfered. The control children got also 100 Kcal excess in addition to their normal diet in plain ice cream. The experimental children got antioxidants as well as 100 Kcal excess in the ice cream in addition to their normal diet. Moreover, deworming the study children cut down the silent blood loss. In addition conversion of beta-carotene to vitamin A added all the advantages of vitamin A including enhancing the immunity, bone metabolism, hematopoiesis cellular health, growth and development and cellular differentiation. Infections have a significant impact on nutrition and growth of children [17]. Vitamin E and other antioxidants also contributed to this end. The long term impact reflects the cumulative effects of multiple infections and the extent of catch up growth. Diarrhea has consistently been seen to have a long term adverse effects on growth [18]. Malaria affects weight but not height, and the causal nature of this association has been confirmed in several malaria prevention trials [19].

It is known that mild zinc deficiency causes growth retardation [20-22]. Also zinc supplementation increases the weight in the children. It is further established that deficiency of vitamin E causes reduced growth [23]. The difference in weight gain in both interventional and control children were found to be 31.47% and 9.65% respectively which was 3.5 times more than control group. Serum level of these antioxidants was corrected for the interventional group up to the optimum level. So, inhibitory factors for normal growth were removed. But the same remained almost static even after intervention in the control group. In addition, appetite in experimental children increased significantly. This might have increased the food intake of the children at home increasing the total energy intake and contributing to significant weight gain.

In the same way baseline mean height (cm) of both the interventional and control groups before and after intervention was measured. The differences of mean height of the groups were (6.76±2.06) and (1.87±0.65) respectively. It was clear from this data that percentage of height increase in interventional and control children were 6.30% and 1.72%, i.e. 3.66 times more in interventional group than the control group and the change was significant.

It is well assumed that the significant gain in weight was due to intervention with antioxidants which acted directly and indirectly by increasing appetite, stimulating immunity, reducing chronic disease conditions and morbidity as well as combating oxidative stress. But contribution of each antioxidant had not been examined separately in this study. It is presumed that zinc played a direct role along with other antioxidants. It was observed that school children who were shorter than their peers had anorexia and were distinguished by having a much lower blood zinc concentration. It could be also such that zinc had some synergistic action with other antioxidants which might need separate confirmation.

It is also known that linear growth failure probably arises from multiple causes, such as maternal malnutrition on fetal growth, alterations of fetal psychosocial conditions, frequent infections and poor dietary intake [24]. Some of the at risk nutrients have a direct role in linear growth like proteins, zinc, vitamin A, calcium phosphorous and secondary impact (e.g. iron, zinc and riboflavin) by inducing impairment of appetite [25].

So, in this study experimental children had enhanced linear growth due to supplementation of zinc, availability of vitamin A because of the supplementation of its precursor (beta-carotene) by the direct role of these 'at risk nutrients'. Secondary impact also contributed to enhance linear growth by improving appetite and increased iron, zinc and riboflavin intake in food.

Triceps skin-fold thickness of the study children was recorded both as baseline and throughout intervention program. The difference in both interventional and control groups was found to be 15.64% and 3.18% respectively, meaning a five times more increase of TSF in the interventional group which was significant statistically. Again a few contributing factors are coming up to explain why such an augmented increase in TSF took place. It was due to the combined effects of antioxidants given to the children in fortified ice cream. This did not happen in case of control children due to the lack of antioxidants in their ice cream. All the antioxidants including zinc contributed in the same way as in weight gain or height increase.

Mid-upper arm circumference (MUAC) was assessed simultaneously in both the groups of study children before and during the whole period of intervention. It was found that the interventional and control children had a baseline mean MUAC (cm) increase as 2.41±0.60 i.e. 13.65% and 0.58±0.22 i.e. 3.60% respectively. The differences of changes in both interventional and control children were significant statistically. The changes in interventional children were four times larger than that in control children. The possible reasons as already discussed are increased appetite, increased immunity, decreased morbidity, reduced anorexia, well controlled oxidative stress etc. The antioxidants contributed in the same way as they contributed in case of other anthropometric growth parameter. It is presumed that antioxidants acted synergistically potentiating the actions of one another.

5 Conclusion

This interventional study evaluated the serum level of beta-carotene, vitamin E, vitamin C and zinc at the beginning and on completion of intervention of the underprivileged preschool children. In respect of all the antioxidants under consideration the base line level is almost similar in both interventional and control groups and it was subnormal in them. But significant changes are seen in interventional group who were fed with antioxidants in recommended doses and serum level resumed back to normalcy but no such changes are seen in the control group who were not given antioxidants. In final evaluation of growth it is seen that significant weight and height gain have taken place in interventional study children but not in control children. Similarly triceps skin-fold thickness and MUAC have also increased significantly in the interventional but not in the control children. So, it is well understood that overall growth of interventional group is achieved well and antioxidants are the contributing factors to it.

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