

Low consumption of fruit and vegetables, and markers of oxidative stress in children with Down syndrome

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Background – Epidemiological, in vitro and in vivo evidence suggests that oxidative stress is elevated in children with Down syndrome. Other studies have indicated that consumption of diets with a high content of fruit and vegetables resulted in a significant reduction in markers of oxidative cellular damage to DNA and lipids in individuals without Down syndrome. **Aim –** We investigated the frequency of consumption of fruit and vegetables by children with Down syndrome and the influence of variations in the level of this consumption on biomarkers of oxidative stress. **Methods –** Frequency of consumption of fruit and vegetables by Iranian children with Down syndrome were recorded over 7 days by the children's parents. Serum malondialdehyde (MDA) and urinary 8-Hydroxydeoxyguanosine (8-OHdG) were also measured. **Results –** Fruit consumed by children with Down syndrome was only 26% of the recommended daily servings (RDS) and vegetable consumption was only 4% of the RDS. Fruit and vegetable consumption was not related to socio-economic status. No significant relationships were found between consumption of fruit and vegetables with markers of oxidative damage. **Conclusion –** Consumption of fruit and vegetables

as an essential part of healthy diet was very low in children with Down syndrome. This seemed unrelated to the cost and availability of these food items, but could be related to the difficulties patients with Down syndrome report chewing. Parents of children with Down syndrome should be encouraged to give more fruit and vegetables to their children and choose soft fruits and preparation methods that make them easier to consume.

Introduction

Down syndrome, or trisomy 21, is the most common chromosomal abnormality in humans. In developed countries incidence of Down syndrome is about one in 800 to one in 1000 live births^[REF 1]. In Iran the age of marriage is increasing and abortion is illegal, so the incidence of Down syndrome is higher at around one in 650-700 live births^[2]. Down syndrome is characterized by several clinical features and metabolic disturbances including mental retardation, immunodeficiency, cataracts, leukemia, endocrine alterations and early ageing. There is considerable literature suggesting a major role for oxidative stress in pathology of Down syndrome^[3-8]. Oxidative stress is defined as an imbalance between production of oxygen-derived free radicals and their removal by antioxidants^[9].

To delay the onset of clinical symptoms in the Down syndrome population an increase in intake of antioxidants has

been recommended by several researchers^[10-14]. Although a randomized controlled trial of a combined antioxidant treatment including vitamins C and E did not produce any developmental benefits^[15], the levels of antioxidants used in this trial were rather low (100% of RDA for vitamin E and 200% for vitamin C). Many free-radical scavengers are naturally present in fruit and vegetables (e.g. as carotenoids and flavonoids)^[16]. Moreover, epidemiological studies have reported an inverse relationship between consumption of fruit and vegetables and markers of oxidative damage to DNA and lipids^[17-19]. Consumption of fruit and vegetables may then be an important natural protective mechanism against oxidative stress in patients with Down syndrome, and low consumption may be in part responsible for their observed elevated levels of oxidative stress. The objective of the present study was to assess the frequency of fruit and vegetable intake in children with Down syndrome and correlate variation in this intake with biomarkers of oxidative stress.

Methods

The study group consisted of 88 children with Down syndrome aged 7-12 years. All children in the study group were pupils selected from 12 Special Education centers for Mentally Handicapped Children in different districts of Tehran, with differing socio-economic status. The children were evaluated by a pediatrician before enrollment in the study had no associated anomalies or disease. In all the children,

Down syndrome diagnosis was confirmed by cytogenetic analysis, which identified subjects with regular trisomy 21. Children lived with their parents and were not on any medication and supplementation therapy. The study was approved by the Ethics Committee of Tehran University of Medicine Sciences in accordance with Helsinki Declaration and guideline of Iranian Ministry of Health and Medical Education (IRB 2507). Written informed consent was obtained from all parents.

Information about the frequency of consumption of fruit and vegetables by the children was obtained by their mothers recording all fruit and vegetable consumption by their children for a week. The mothers were trained in advance to identify standard portion sizes by a dietitian to help them collect records accurately. Portion sizes reported by mothers were translated into 'servings' by a dietitian. Data on working status and level of education and locality where the participants lived were collected by interview with the mothers.

We measured malondialdehyde (MDA) in serum and 8-Hydroxydeoxyguanosine (8-OHdG) in urine samples as markers of oxidative stress.

To determine serum MDA concentrations, 5ml fasting blood were collected from children. We didn't collect blood from 12 children because of 9 of them were afraid to give blood, and in 3 of the children we couldn't find a vein to take the blood. Serum was drawn after at least 30 minutes of clotting by centrifugation at 2500g for 15 minutes. The determination of MDA levels was performed by method of Satho^[20]. In this method the reaction of MDA with Thiobarbituric acid (TBA) creates a complex which is determined spectrophotometrically. Briefly serum samples were mixed with trichloroacetic acid (TCA) (20%) and the precipitate was dissolved in sulfuric acid (0.05M). TBA (0.02% in sodium sulfate 2M) was added and heated for 30 minutes in boiling water bath. TBA reactive substances (TBARS) adducts were extracted by n-butanol and absorbance was measured at 532nm by UV-160-A Shimadzu double beam spectrophotometer (Kyoto, Japan).

Ten millilitre spot morning urine samples were collected in polypropylene specimen tubes, since it proved difficult to collect 24-hour urine samples in chil-

dren with Down syndrome. Thompson et al^[17] indicated 24-hour average urinary levels were not statistically different from first voids, so we decided use first morning voids rather than 24-hour collections. 8OHdG in urine samples was assessed using an enzyme-linked immunosorbent assay (ELISA) kit (8OHdG Quantitation, Cell Biolabs, Inc. San Diego). The ELISA assay was performed according to the manufacturer's instructions. Urine samples were centrifuged at 3000g for 10 minutes then supernatant was diluted with phosphate buffered saline. A 50µl aliquot of the primary antibody and 50µl of the digested DNA and diluted urine samples were added to a microplate that had been precoated with 8OHdG then they were incubated at room temperature for 1 hour on an orbital shaker after which the plate was washed 3 times thoroughly with 250µl washing solution. Each of the wells on the plate was then incubated at room temperature for 1 hour with 100µl horseradish peroxidase (HRP)-conjugated second antibody and subsequently washed with 250µl washing solution. In the next step 100µl enzyme substrate solution was added and the plates were incubated at room temperature for 15 minutes on an orbital shaker. The enzyme reaction was stopped by adding 100µl of stop solution (1M phosphoric acid) for about 3 minutes, after which the absorbance at 450nm could be read using a Benchmark Microplate Reader. The above procedures were performed under dark conditions. Concentration of 8OHdG was calculated from a standard curve.

Results are expressed as mean ± SD. For comparison of the groups, Student's t-test and analysis of variance (ANOVA) were performed. Pearson's correlation was used to establish correlations between variables. A P-value less than 0.05 was considered significant. Analyses were carried out using the Statistical Package for Social Sciences (SPSS 13.00 for windows).

Results

The mean age of the children with Down syndrome was 11.26 ± 2.65 years, and 39.8% of them (27/88) were girls and 60.2% (51/88) were boys. The age difference between two sexes was not statistically significant ($P > .05$). Based on the 7-day fruit and vegetable diary records, mean consumption of fruit and vegetables was 4.6 servings/ week for fruit and 0.74 servings/week for vegetables. Fifty eight (65.9%) children didn't consumed any type of vegetable, and consumption of the others was normally less than one serving per day. Frequency of consumption of fruit in 45 children (51.1%) was less than one serving per day. Consumption of fruit and vegetables by children with Down syndrome were not significantly related to socio-economic status.

The mean serum level of MDA was 2.91 ± .73 nmol/ml in the children with Down syndrome. No statistically significant differences were found in serum MDA levels between the two sexes. The urine levels of 8OHdG in boys were significantly higher than in girls ($3.22 \pm .41$ ng/ml vs $2.93 \pm .57$ ng/ml, $P < .01$) (TABLE1). There was no significant correlation between age and the levels of MDA in serum and 8OHdG in urine. There was also no significant relationship between consumption of fruit and vegetables and the markers of oxidative stress.

Discussion

There is increasing evidence that elevated oxidative stress is involved in the pathology of Down syndrome. In theory, therefore, using antioxidant nutrients to scavenge oxygen - derived free radicals may reduce or delay some of the symptoms of Down syndrome. Fruit and vegetables are prominent sources of essential nutrients, many of which have antioxidant activity. Fruit and vegetable intake has been previously established to be inversely correlated with markers of

Sex	age	MDA (nmol/ml)	8OHdG (ng/ml)	fruit/week	vegetables/ week
Boys	10.98±2.86	2.98±.90	3.22±.41	4.89±2.70	.64±1.30
Girls	11.54±2.26	2.78±.56	2.93±.57*	4.26±2.44	.89±1.18
Total	11.20±2.64	2.90±.79	3.11±.50	4.64±2.60	.73±1.22

Table 1 | Age, levels of serum MDA, urine 8OHdG and frequency of consumption of fruit and vegetables in children with Down syndrome.

* $P < 0.01$

oxidative stress^[21]. High intakes of fruit and vegetables may then be protective for patients with Down syndrome^[22]. The recommended daily servings (RDS) of fruit and vegetables for school-age children are 2-3 servings for each of them. In the present study consumption of fruit and vegetables by children with Down syndrome were respectively only 26% and 4% of the RDS. This contrasts the intake of a sample of over 20,000 children studied from across Iran, that did not have Down syndrome, which was substantially higher at around 2 servings per day^[23]. No statistically significant differences were found in consumption of fruit and vegetables by children with Down syndrome from different socio-economic groups. It seems therefore that financial cost and availability of fruit and vegetables was probably not a major factor influencing the low level of consumption of fruit and vegetables by children with Down syndrome. Chewing problems are common in people with Down syndrome^[24] and this problem may lead to decrease in the desire to eat fruit and vegetables in children with Down syndrome. In preparing fruit and vegetables for children with Down syndrome it is important to make sure they are soft enough for the children to easily chew them. There may be additional advantages of elevating consumption of fruit and vegetables in these subjects. Both obesity^[25] and constipation^[26] are common problems in children with Down syndrome. Fresh fruit and vegetables not only contain natural antioxidants but also provide non-starch polysaccharides and have low energy density leading to greater satiety. Regular and sufficient consumption of fruit and vegetables is recommended for the prevention and treatment of both constipation and obesity^[27,28]. Parents of children with Down syndrome should be encouraged to increase the amounts of fruit and vegetables they give to their children.

Our data did not reveal any significant relationship between consumption of fruit and vegetables with markers of oxidative stress in children with Down syndrome. Our findings are in agreement with that reported by Jovanovic et al^[22]. In studies where consumption of fruit and vegetables has been correlated with a significant decrease in oxidative stress markers the amounts of fruit and vegetables con-

sumed by subjects have been several times greater than the RDS. Consequently, it is not surprising that we did not find any significant relationship between intake of fruit and vegetables with markers of oxidative stress. The absence of such a relationship in this sample does not mean that improving the diets of children with Down syndrome would be ineffective as a method to reduce oxidative stress. However, controlled intervention trials are needed to establish if this is the case.

In summary these data show that consumption of fruit and vegetables in children with Down syndrome is very low relative to the RDS for this age group. This may contribute to their pathological symptoms including oxidative stress, constipation and obesity. Low consumption of fruit and vegetables may be related to the difficulties children with Down syndrome have chewing. Choice of appropriate soft vegetables and preparation methods to make these foods easier to chew may help children with Down syndrome increase their intake of these important dietary components. Parents of children with Down syndrome should be encouraged to feed their children more of these food types.

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