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Oxidative stress status in nutritionally stunted children

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KEYWORDS

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Abstract *Introduction:* Oxidative stress is the imbalance between pro-oxidants and antioxidants resulting in irreversible cell damage.
Objective: To assess the oxidative stress status in a sample of Egyptian malnourished stunted children and investigate the relations between oxidative stress markers and anthropometric measurements.
Patients and methods: This cross sectional descriptive analytical study was carried out on 50 malnourished stunted children (28 males and 22 females), aged from 6–9 years and 50 healthy age and sex-matched controls. Blood oxidative stress biomarkers including catalase (CAT), superoxide dismutase (SOD) malondialdehyde (MDA), plasma glutathione (GSH), total plasma proteins, total anti-oxidant capacity (TAC), copper (Cu), zinc (Zn), and vitamin C were measured in patients and controls. Socio-economic status was assessed for patients. Body weight and height were measured and body mass index (BMI) was calculated. Patients were classified according to their height for age Z-scores (HAZ) into moderate and severe stunted.
Results: Nutritionally stunted children showed significantly lower levels of the blood oxidative stress biomarkers including, CAT, SOD, plasma GSH, total plasma proteins, Cu, Zn and vitamin C and significantly higher levels of MDA compared with controls ($p < 0.001$). There was significant difference in plasma levels of Vitamin C and Zn between patients with different social levels. No significant relationships were found between the degree of stunting and oxidative markers.
Conclusions: Nutritionally stunted children had an increased oxidative stress and decreased antioxidant defense system compared with healthy controls. Oxidative stress, malnutrition and low social level might play an important role in the pathogenesis of stunting.

Introduction

Stunting, defined as "the gaining of insufficient height relative to age"[1] is a major public-health problem in underdeveloped and developing countries.[2–4] The WHO (2000) report, demonstrated that 215 million children were stunted.[5]

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Malnutrition is one of the major causes of mortality and accounts for fifty percent deaths in children less than five years of age. Therefore, malnutrition represents one of the most severe health problems in the world.[6] Malnutrition is a major public problem in many parts of the world. One of the clearest manifestations of prolonged malnutrition in childhood is compromised stature, it has been estimated that 43% of children worldwide are stunted.[7] Stunting is generally used as an indicator of long-term chronic nutritional deficiency. Child stunting has been extensively studied in developing countries.[8–10]

Oxidative stress can occur due to overproduction of reactive oxygen species (ROSs), decrease in antioxidant defenses or a combination of these factors. Free radicals and other reactive species are produced in the body primarily as a result of oxygen consumption. Antioxidants (glutathione, vitamins A, E and C, selenium, zinc etc.) and antioxidant enzymes (e.g. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) exert synergistic effects in scavenging free radicals. There has been growing evidence showing that malnutrition (e.g. dietary deficiency of protein, selenium or zinc) gives rise to oxidant stress and cell injury.[11–13] The aim of this study was to evaluate the oxidative stress status in a sample of malnourished stunted children and to investigate the possible relations between oxidative stress markers and anthropometric parameters.

Patients and methods

Patients

The present study is a descriptive and analytical study which included pre-pubertal 50 malnourished stunted children (28 males and 22 females) and 50 healthy age and sex matched controls. Stunting was defined as height-for-age more than 2 SDs below the median value of the WHO International Growth References height for age.

Inclusion criteria included

School children aged 6–9 y, height more than 2 SDs below the WHO age and sex specific mean. Both sexes were included. Control cases were of the same age group with normal height for age and sex.

Exclusion criteria included

Children with endocrinal or chromosomal causes of stunting or with chronic debilitating diseases and cases with skeletal dysplasias such as achondroplasia and syndromatic short stature.

Ethical approval: was obtained from the "Ethics Committee" of the National Research Centre. Written informed consent was obtained from the parents after explanation of the aim of the study and its possible benefits for identifying the cause of the growth delay of their children and other children who have the same condition.

Assessment of the social status: was carried out according to Park & Park[14] scoring, using parental education and occupation. The score was classified into low (score <8), middle (score 9–18) and high (score 19–28).

Dietary assessment: Methods used for measuring food consumption of the surveyed children were classified into two major groups.[15] The first group, known as the quantitative daily consumption method, consisted of recalls or records designed to measure the quantity of foods and beverages consumed over one day period "Twenty-four-hour recall" method. The second group of methods included the dietary pattern and food frequency questionnaire.

A Twenty-four hour recall method

In this method parents of every surveyed child were asked to recall the exact foods and beverages intake during the previous 24 h period. Quantities of foods and beverages consumed were estimated in household measures and grams.

The obtained information included eating events in sequence beginning with first eating event of the day, specifying each event whether major or minor and recording food items in each event.

Adequacy of the diet consumed was assessed by comparing the energy and nutrient intake of the person with his recommended dietary allowances "RDA" according to FAO/WHO/UNU 2001, 2002 and 2007.[16–18]

A food coding system was used, based on 2 digits denoting the food group, 2 digits denoting the food item and 2 digits denoting the method of preparation.

The conversion of grams of foods and beverages to energy and nutrients was carried out by computer program based on energy and nutrient database developed from the Food Composition Tables, NNI.[19]

Analysis was based on

Patients were divided according to energy, protein and micronutrient% intake of the recommended dietary allowances (RDA) into two groups: group 1 = patients with deficient dietary intake (less than 50% of RDA) for each nutrient and group 2 = patients with accepted dietary intake ($>50\%$ of the RDA).

B-Dietary pattern "Food Frequency Questionnaire"[15]

This method was used to obtain qualitative descriptive information about usual food and beverages consumption pattern for the child per day and per week.

This questionnaire includes:

- Energy foods:
  Cereals and its products – oils and fats – sugar and sweets
- Tissue building foods:
  Meat, chicken, fish, eggs, legumes, milk and its products
- Protective foods:
  Vegetables and fruits
- Beverages:
  Tea, coffee, carcadeh, fenugreek, cola and fruit juice.

Anthropometric measurements included

Body weight and height were measured using standardized equipments and techniques, and following the recommendations of the International Biological Program[20] and body mass index (BMI) was calculated as weight (kg) divided by the square of the height (meters). Anthropometric parameters were expressed in terms of standard deviation scores (Z-scores), using the WHO standard growth curves[21] as reference population. Z-score was calculated according to Bruce[22] Patients were

categorized according to their degree of stunting into two groups; the first group ($n = 35$; 70%) as having moderate stunting (height between −2SD and −3SD), while the second group ($n = 15$; 30%) as having severe stunting (height ⩽ −3SD). We compared the levels of oxidative stress biomarkers in both groups.

Laboratory investigations

Blood level of some enzymatic antioxidants (CAT) was estimated according to the methods of Aebi,[23] SOD was estimated according to the methods of Nishikimi et al.,[24] plasma level of none enzymatic antioxidants (GSH) was estimated according to the methods of Satoh,[25] total plasma proteins were estimated according to the methods of Beutler et al.,[26] total antioxidant capacity (TAC) was estimated according to the methods of Hayakawa and Jap[27] as well as plasma levels of Cu were estimated according to the methods of Koracevic et al.,[28] Zn was estimated according to the methods of Harris and Ray[29] and vitamin C was estimated according to the methods of Eden and Green[30] Additionally, blood level of MDA as a marker of free radical mediated peroxidative tissue damage was estimated according to the methods of Gornal et al.[31] All the above mentioned parameters were measured in malnourished stunted school children and healthy controls.

Statistical analysis

Statistical analysis was performed using SPSS (version 16). Results are presented as mean ± SD. The Student's *t*-test was used to assess differences in the means of continuous variables. For comparing blood parameters of patients according to the social standard, we applied one-way analysis of variance (ANOVA). *P* value <0.05 was used as a threshold of significance.

Results

The mean age for male patients was 7.50 ± 0.96 and for female patients was 7.68 ± 1.09.

Table 1 shows comparison between patients and age and sex matched controls concerning the oxidative stress biomarkers. Malnourished stunted children displayed significantly decreased levels of the blood oxidative stress biomarkers including, anti-oxidant enzymes Catalase, Superoxide dismutase, plasma Glutathione, total plasma proteins and TAC as well as plasma Cu, Zn and vitamin C than controls ($p < 0.01$) in concomitant with significant increased level of blood level of MDA ($P < 0.01$).

Table 2 shows comparison between cases with moderate and severe stunting regarding the oxidative stress indices. Analysis showed no statistical significant difference in levels of oxidative stress markers between the two groups.

Table 3 shows the means of oxidative stress markers and anthropometric parameters in patients according to their social classes. Significant difference was observed in the level of Zn ($p = 0.04$), there was significant difference in plasma level of Zn between patients with low and those with high social score ($p = 0.01$). Moreover, there is significant difference also in plasma level of Vit.C ($p = 0.03$) of patients according to their socioeconomic standard. The difference was also significant between patients with low and those with high social score ($p = 0.01$). Furthermore, there was significant difference between patients with middle and those with high social score

Table 1 Comparison of oxidative stress markers of patients and healthy controls.

	Patients ($N = 50$)		Control ($N = 50$)		*p*
Variable	Mean ± SD	Range	Mean ± SD	Range	
Catalase (U/mg Hb)	3.77 ± 0.16	3.35–3.97	5.49 ± 0.40	4.86–6.1	0.001
SOD (U/mg Hb)	1.12 ± 0.06	1–1.21	2.11 ± 0.08	2–2.4	0.001
MDA (nmol/g Hb)	0.59 ± 0-06	0.5–0.7	0.33 ± 0.04	0.26–0.39	0.001
TAC (mmol/L)	1.56 ± 0.150	1.23–1.84	2.14 ± 0.05	2–2.25	0.001
GSH (mg/dL)	46.62 ± 3.63	39.9–52.97	55.66 ± 0.88	54.22–58.53	0.001
Protein (g/dL)	5.85 ± 0.45	5–6.5	6.49 ± 0.33	5.98–7.5	0.001
Cu (μg/dL)	59.96 ± 4.27	51.16–67.42	73.98 ± 1.74	70–77.5	0.001
Zn (μg/dL)	54.01 ± 2.45	46.71–58.45	65.34 ± 1.38	63–69	0.001
Vit C (mg/dL)	0.96 ± 0.13	0.72–1.25	1.49 ± 0.09	1.33–1.67	0.001

Table 2 Comparison of oxidative stress markers in patients according to the degree of stunting.

Variable	Moderate Patients with −2SD > Ht. > −3SD ($N = 35$)	Severe Patients with Ht. ⩽ −3SD ($N = 15$)	*P*
Catalase (U/mg Hb)	3.77 ± 0.17	3.77 ± 0.16	1.00
SOD (U/mg Hb)	1.12 ± 0.07	1.12 ± 0.06	0.99
MDA (nmol/g Hb)	0.59 ± 0.06	0.60 ± 0.05	0.57
TAC (mmol/L)	1.55 ± 1.55	1.57 ± 0.15.039	0.81
GSH (mg/dL)	47.16 ± 3.37	45.38 ± 4.01	0.15
Proteins (g/dL)	5.85 ± 0.47	5.85 ± 0.43	0.96
Cu (μg/dL)	60.50 ± 4.53	58.68 ± 3.39	0.13
Zn (μg/dL)	53.73 ± 2.64	54.65 ± 1.83	0.17
Vit C (mg/dL)	0.95 ± 0.95	0.99 ± 0.10	0.28

Table 3 Comparison of oxidative stress markers and anthropometry of patients according to their socioeconomic classes.

SES OS markers	Low (range) ($N = 22$)	Middle (range) ($N = 20$)	High (range) ($N = 8$)	P
Catalase (U/mg Hb)	3.77 ± 0.18 (3.35–3.96)	3.75 ± 0.16 (3.49–3.95)	3.84 ± 0.09 (3.74–3.97)	0.43
SOD (U/mg Hb)	1.11 ± 0.07 1–1.21	1.12 ± 0.06 1–1.2	1.13 ± 0.04 1.08–1.18	0.74
MDA (nmol/g Hb)	0.58 ± 0.06 0.50–0.70	0.60 ± 0.06 0.52–0.69	0.60 ± 0.06 0.50–0.68	0.46
TAC (mmol/L)	1.57 ± 0.17 1.23–1.84	1.56 ± 0.14 1.34–1.79	1.53 ± 0.11 1.34–1.69	0.89
GSH (mg/dL)	46.24 ± 3.82 39.9–51.9	46.67 ± 3.51 40.39–52.75	47.56 ± 3.63 41.59–52.97	0.68
Proteins (g/dL)	5.7 ± 0.42 5.1–6.5	5.91 ± 0.51 5–6.50	5.83 ± 0.43 5.2–6.40	0.8
Cu (μg/dL)	59.47 ± 4.33 51.16–56.34	6.18 ± 4.32 51.47–67.42	60.72 ± 4.4 54–66.27	0.75
Zn (μg/dL)	53.22 ± 2.55* 46.71–57.81	54.2 ± 2.29 49.44–57.84	55.68 ± 1.7 53.04–58.45	0.04
Vit. C (mg/dL)	0.94 ± 0.11# 0.72–1.17	0.94 ± 0.12 0.74–1.19	1.07 ± 0.17 0.78–1.25	0.03
Z Wt.	−2.31 ± 1.10 −4.45–0.57	−1.78 ± 0.66 −3.36–(−1.02)	−2.42 ± 1.15 −4.8–(−1.12)	0.13
Z Ht.	−3.02 ± 0.83 −5.01–(−2.12)	−2.68 ± 0.54 −4.14–(−2.03)	−2.94 ± 0.94 −4.8–(−2.08)	0.33
ZBMI	−0.36 ± 0.28 −2.88–2.56	.012 ± 0.22 −2.63–1.68	−0.63 ± 0.37 −2.12–0.74	0.36

Abbreviations: SD, standard deviation; SOD, super-oxide-dismutase; MDA, malondialdehyde; TAC, total anti-oxidant capacity; GSH, reduced glutathione; Cu, copper; Zn, zinc; Vit. C, vitamin C; Ht, height; SES, socio-economic status; OS, oxidative stress markers; Wt, weight; BMI, body mass index.

($p = 0.02$). There was no statistical significant difference in the other oxidative stress markers and anthropometric measures of patients as regards their social standard.

Discussion

The etiology of stunting is thought to be multifactorial including inadequate nutrition, sustained infections and poor mother–child interaction.[32–34]

Several mechanisms may lead to oxidative stress in malnourished children. The most important one is the sub-normal intake of nutrients such as carbohydrates, proteins and vitamins, leading eventually to accumulation of ROSs. Reduced concentrations of vitamin A and of the anti-oxidant vitamins C and E together with deficiency of trace elements (selenium, zinc, and copper) were previously reported in children with malnutrition.[11–13] Oxidative stress may be a major underlying cause for both conditions.[35]

The present study showed that malnourished stunted children have significantly lower levels of blood oxidative stress biomarkers including, anti-oxidant enzymes; catalase, SOD, plasma glutathione, total plasma proteins, TAC, Cu, Zn and vitamin C compared with controls ($p < 0.01$). Koracevic et al.[28] stated that, the total anti-oxidant capacity (TAC) of serum is not a simple sum of the activities of various anti-oxidative substances. It is a dynamic equilibrium that is influenced by the interaction between each serum anti-oxidant constituent. It is thought that the co-operation of antioxidants in human blood provides greater protection against attack by free radicals than any anti-oxidant alone. It is well established that any alterations in the oxidant and anti-oxidant balance results in oxidative stress. Our results are in agreement with the results obtained by various authors.[36–41] Moreover, various studies reported decreased anti-oxidant status in malnourished children.[42,43,11,44,12,45,13,46]

The reason for decreased anti-oxidant capacity (TAC) in malnourished children is the increased generation of ROS. As anti-oxidants are involved in fighting against oxidative stress, some amount might be exhausted. Another reason could be due to low SOD activity due to low level of copper and zinc which are co-factors for SOD. The trace element Zn plays an important role as nutritional co-factor. A decrease in Zn status leads to a decrease in the activity of some Zn-dependent enzymes such as SOD, lactate dehydrogenase, glutamate dehydrogenase, pyridoxal phosphokinase and thymidine kinase.[47] Thus, Zn can also modulate protein, energy and nucleic acid metabolism by affecting enzymes containing or requiring Zn for their activities. Zn deficiency in malnourished children would obviously lead to lowered activity of SOD, which ultimately results in lowered TAC.

In our study we also found that MDA concentration in malnourished children was significantly high. Various authors,[12,45,13,46] also found increased lipid peroxidation in malnourished children. These results indicate that the decreased defense status of blood may result in increased lipid peroxidation of all membrane lipids and enhanced concentration of lipid peroxidation products like MDA.

Jain et al.[48] concluded that, the anti-oxidant defense system is adversely affected in protein energy malnutrition (PEM). They also provided evidence that there are two important pathophysiological changes, a weakened antioxidant defense

system and increased lipid peroxidation, occurring in PEM. A dietary deficiency of nutritional anti-oxidants and minerals including Zn, in malnourished children would result in disturbance of the anti-oxidant defense system. Therefore, it would be advantageous to add anti-oxidant nutrients, including Zn, to food supplement in order to achieve normal level of serum Zn to promote growth, enhance clinical recovery and restore immuno-competence.

A study was done in Bangladesh, which proved that micronutrient deficiency such as zinc, folic acid, iron, vitamin B12, and increased lipid peroxidation as indicated by increased level of MDA during pregnancy was associated with small for gestational age (SGA). An increase in oxidative stress has been observed in neonates born small for gestational age (SGA) by malnourished mothers,[49] and in pre-pubertal children born SGA.[50] There was also increased DNA oxidation which was associated with decreasing IGF-1 in early childhood, implying longer-term association with short stature.[51]

In the current study, it was also found that, the plasma level of zinc is affected by the social score of the patients, as there was significant difference in zinc level between patients with low and those with high social score being more in high social class. Moreover, we found that there is significant positive correlation between plasma level of zinc and social class.

Furthermore, we also found that the level of vitamin C is significantly different according to social class but it was more in high than low social class. It was also more in high than middle social class. So we can conclude that the higher the socioeconomic class the less the oxidative stress.

Semba et al.[8] looked at indicators of child growth, parental education, social and economic status in nearly 600,000 families in Indonesia, and almost 400,000 in Bangladesh. They found that 1/3 of children younger than 5 y were growth stunted, in Indonesia. They found also that greater education of the mother reduced the child stunting by 4.4% in urban areas and 5% in rural areas. Education of the father decreased the stunting by 3%. In Bangladesh, they found that 1/2 the children are stunted, mother's education reduced the stunting by 4.6% while father's education decreased the risk of stunting by 2.9% in rural and 5.4% in urban areas. The authors explained these findings by increased use of ''health-promoting'' behaviors by educated parents like getting their kids vaccinated and giving them vitamin supplements. So, they concluded that educated parents are less likely to have stunted kids.

The decreased levels of antioxidants found in our study, could be attributed to the depletion in blood anti-oxidant stores due to their consumption during free radical scavenging in response to ROSs production. It could be also attributed to a decrease in the supply of dietary antioxidants due to malnutrition. The second cause for oxidative stress in malnutrition may be due to a non-specific chronic activation of the immune system as a result of chronic inflammation. Many conditions leading to malnutrition and wasting may also cause inflammation.

Conclusions

Malnourished stunted children had an increased oxidative stress and decreased antioxidant defense system compared with healthy controls. Oxidative stress and low social level might play an important role in the pathogenesis of stunting among malnourished stunted children.

Conflict of interest

None declared.

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