Impact of nutritional rehabilitation on enzymatic antioxidant levels in protein energy malnutrition

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تأثير التأهيل التغذوي على مستويات مضادات الأكسدة الإنزيمية في سوء التغذية بالبروتين والطاقة

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الخلاصة: من أجل تقييم دور مضادات الأكسدة الإنزيمية في التسبُّب بسوء التغذية بالبروتين والطاقة وتأثير التأهيل التغذوي عليه، درسنا 30 طفلاً يعانون من سوء التغذية بالبروتين والطاقة العمر المتوسط 10.63 ± 4.39 شهراً: وكان 10 أطفال منهم مصابين بالسِغِل؛ وكان 8 منهم مصابين بالكواشيوركور؛ وكان 12 طفلاً مصاباً بالسِغِل والكواشيوركور معاً إلى جانب 15 من الأطفال الشواهد. وقد أجري فحص سريري واستقصاء مختبري، وقد تضمن ذلك تقدير إنزيم ديسموتاز فوق الأكسيد وإنزيم بيروكسيداز الغلوتاثيون، قبل وبعد التأهيل التغذوي. وقد نقص كل من هذين الإنزيمين بشكل كبير في كلّ الأطفال المصابين بسوء التغذية إذا ما قورنوا بالمجموعة الشاهدة، وزاد هذان الإنزيمان بشكل كبير بعد التأهيل التغذوي. هذه العلاقة الوثيقة توضح أنّ مضادات الأكسدة يمكن أن تقدَّم للمصابين بسوء التغذية بالبروتين والطاقة أثناء التأهيل التغذوي لهم لإنقاص المراضة ومعدل الوفيات.

ABSTRACT To assess the role of enzymatic antioxidants in the pathogenesis of protein energy malnutrition (PEM) and the effect of nutritional rehabilitation, we studied 30 infants with PEM (mean age 10.63 ± 4.39 months: 10 marasmic; 8 with kwashiorkor; 12 with marasmic kwashiorkor) and 15 controls. All underwent clinical examination and laboratory investigations, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) estimation before and after nutrition rehabilitation. SOD and GPx were significantly lower in all malnourished infants compared to controls, and significantly increased after nutritional rehabilitation. These significant correlations suggest that antioxidants could be introduced during PEM nutritional rehabilitation to decrease morbidity and mortality.

Impact de la réhabilitation nutritionnelle sur les niveaux des antioxydants enzymatiques dans la malnutrition protéino-énergétique

RESUME Afin d'évaluer le rôle des antioxydants enzymatiques dans la pathogenèse de la malnutrition protéino-énergétique et l'effet de la réhabilitation nutritionnelle, nous avons étudié 30 nourrissons atteints de malnutrition protéino-énergétique (âge moyen 10,63 ± 4,39 mois : 10 atteints de marasme; 8 de kwashiorkor; 12 de kwashiorkor marastique) et 15 témoins. Tous ont été soumis à un examen clinique et des analyses de laboratoire, y compris l'évaluation de la superoxyde dismutase et de la glutathion-peroxydase avant et après la réhabilitation nutritionnelle. La superoxyde dismutase et la glutathion-peroxydase étaient significativement réduites chez tous les nourrissons malnutris par rapport aux témoins, et significativement élevées après la réhabilitation nutritionnelle. Ces corrélations significatives laissent penser que les antioxydants pourraient être introduits pendant la réhabilitation nutritionnelle en cas de malnutrition protéino-énergétique afin de réduire la morbidité et la mortalité.

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Introduction

In Egypt, protein energy malnutrition (PEM) is quite common and stands as a major threat to infant health, growth and development. In recent years, reactive oxygen species (ROS) have been implicated in the pathogenesis of many conditions including PEM-associated oedema and anaemia [1]. Oxidative stress in PEM infants may result from either increased production of free radicals [2] or depletion of the antioxidant defence mechanisms [3]. Disequilibrium in the balance between oxidants and antioxidants has been reported as a possible etiology for kwashiorkor [4]. This imbalance can be used as a prognostic factor in children with PEM [5].

Superoxide dismutase (SOD) is an enzyme that acts as a potent superoxide scavenger [6]. Glutathione peroxidase (GPx) on the other hand is the major enzyme that removes H_2O_2 generated by SOD in cytosol and mitochondria [7].

This study was designed to assess the role of enzymatic antioxidants, represented by SOD in erythrocytes and GPx in whole blood, in the pathogenesis of PEM. To emphasize the role of nutrition rehabilitation we reassessed the cases studied and controls after 30 ± 7 days.

Methods

We studied 45 infants from the Paediatric Hospital, Ain Shams University, Cairo, Egypt — 30 infants with PEM (16 males and 14 females; mean age 10.63 ± 4.39 months) and 15 healthy matched controls.

The 30 patients with PEM were categorized according to the Wellcome classification [8] into three groups. The first group included 10 marasmic infants (5 males, 5 females; mean weight 4.20 ± 1.11 kg; mean age 9.60 ± 5.65 months). The second group included 12 kwashiorkor infants (7 males, 5 females; mean weight 5.67 ± 1.40 kg; mean age 9.58 ± 7.72 months). The third group comprised 8 marasmic kwashiorkor infants (4 males, 4 females; mean weight 4.86 ± 1.47 kg).

The infants were also divided into three subgroups according to the new classification proposed by Gernaat and Voorhoeve [9]. This classification is based on the presence or absence of oedema and wasting measured as a Z-score of weight-for-height (difference between the individual's actual measurement and the median value of an age and sex matched reference population divided by the standard deviation of the reference population). Wasting is here defined as a Z-score of weight-for-height < −2 SD. In a normally distributed reference population, 95% of cases fall by definition within the range of 2 SD from the median. Thus, 2.5% of the reference population falls below −2 SD (Table 1). The marasmic infants had a mean Z-score of −2.10 ± 1.57, the kwashiorkor infants had a mean Z-score of −1.0 ± 0.41 while that of the marasmic kwashiorkor infants was −4.13 ± 0.67. The 15 healthy controls showed normal anthropometric measures for their age and sex and a mean Z-score of 0.69 ± 1.06 and mean age of 10.63 ± 5.32 months.

Verbal consent was obtained from the infants' parents. A detailed history was obtained from both patients and controls, and a thorough clinical examination made. Dietetic history and the duration, type and degree of malnutrition by different anthropometric measurements were explored. Laboratory investigations included complete blood count (CBC), C-reactive protein (CRP), total serum proteins, serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum

Table 1 **The Gernaat and Voorhoeve classification of acute protein energy malnutrition (PEM)**

Weight-for-height Z-score	Oedema present	Oedema absent
Z > –2 (no wasting)	Kwashiorkor	No acute PEM
Z < –2 (wasting)	Marasmic kwashiorkor	Marasmus

Source: [9].

creatinine (Cr) and blood urea nitrogen (BUN), SOD in erythrocytes and GPx in whole blood (both supplied by Randox Laboratories Limited, United Kingdom).

After 30 ± 7 days of a supervised nutritional programme according to World Health Organization guidelines [*10*], re-evaluation of the patients was performed using the same pre-intervention measurements and investigations. In the first week, supervised feeding was started immediately after any life-threatening and emergency conditions were brought under control.

Total caloric intake was 80–100 kcal/kg per day, keeping in mind the continuity of breastfeeding in cases of breastfed infants. The diet given was low in protein, fat, and sodium and high in carbohydrates, as almost all severely malnourished infants have infections, impaired liver and intestinal function and problems related to electrolyte imbalance. Table 2 shows the recommended daily nutrient intake during the initial stage of treatment.

The rehabilitation stage followed after the return of the infant's appetite. The caloric intake was increased to 150–200 kcal/kg per day with an increase in quantity and decrease in frequency. A high protein diet was given and vitamins and minerals (potassium, magnesium and zinc) were continued in increased amounts. Iron was given during this stage to treat the anaemia present. The infants remained in the hospital for the first part of this rehabilitation phase (3 weeks after admission), and were then followed up in the outpatient clinic for Nutrition Rehabilitation.

Statistical analysis of the results was carried out using *SPSS*.

Results

SOD and GPx levels were significantly decreased in the 30 patients and in each subgroup before nutrition rehabilitation when compared to the control group (Tables 3 and 4), with no significant difference between the subgroups. After nutrition rehabilitation, both enzymes were significantly increased in all malnourished infants and in each subgroup (Tables 3 and 4) to the extent that GPx no longer showed any significant difference with the controls (Table 4). However, despite the obvious improvement, SOD levels were still significantly lower than those of the controls after nutrition rehabilitation in all patients and in each subgroup (Table 3).

On applying correlation tests between the rate of change of either SOD and GPx and the rate of change of each of the Z-scores, Hb % and albumin level, a significant positive correlation was found in all malnourished infants (Table 5). However, a

Table 2 **Desirable daily nutrient intake during the initial treatment phase for protein energy malnutrition, according to World Health Organization guidelines**

Nutrient	Amount/kg of body weight
Water	120–140 mL
Energy	100 kcal
Protein	1–2 g
Electrolytes	
Sodium	1.0 mmol (23 mg)
Potassium	4.0 mmol (160 mg)
Magnesium	0.6 mmol (10 mg)
Phosphorus	2.0 mmol (60 mg)
Calcium	2.0 mmol (80 mg)
Trace minerals	
Zinc	30.0 µmol (2.0 mg)
Copper	1.5 µmol (0.3 mg)
Selenium	60.0 nmol (4.7 µg)
Iodine	0.1 µmol (12 µg)
Water-soluble vitamins	
Thiamine (vitamin B_1)	70.0 µg
Riboflavin (vitamin B_2)	0.2 mg
Nicontinic acid	1.0 mg
Pyridoxine (vitamin B_6)	70.0 µg
Cyanocobalamin (vitamin B_{12})	0.1 mg
Folic acid	0.1 mg
Ascorbic acid (vitamin C)	10.0 µg
Pantothenic acid (vitamin B_5)	0.3 mg
Biotin	10.0 µg
Fat-soluble vitamins	
Retinol (vitamin A)	0.15 mg
Calciferol (vitamin D)	3.0 µg
α tooophorol (vitamin E)	2.2 mg
Vitamin K	4.0 µg

Source: [*10*].

significant negative correlation was detected between the rate of change of both enzymes and the rate of change of total leukocyte count and ALT (Table 5).

Table 6 and Table 7 demonstrate the comparison between the different anthropometric measurements and laboratory parameters before and after nutrition rehabilitation. The anthropometric measurements, total leukocyte count, Hb %, total proteins and serum albumin showed significant differences, while ALT and Cr showed no significant changes. Kidney functions (Cr and BUN) showed values within the normal reference range for equivalent age and sex, both before and after nutrition rehabilitation [*11*].

Discussion

The results of the present study show that the SOD level was significantly decreased in all malnourished infants and in each subgroup when compared to the controls, with no significant difference between the subgroups. Similar findings have been reported by Golden and Ramdath [*5*], where SOD deficiency in PEM was explained by either consumption in dismutating reactions where ROS are removed by SOD, or as being secondary to deficient levels of copper or zinc, both known to be integral parts of copper–zinc SOD. This hypothesis is supported by the report of Ashour et al. who found that plasma copper was significantly decreased in PEM [*12*].

Our finding of significantly improved SOD levels following nutritional rehabilitation accords with a study by Golden and Ramdath [*5*]. The consumed SOD moves closer to normal levels with the reverse of PEM pathology and micronutrient supplementation during the rehabilitation programme. The short duration of rehabilitation may explain why, in spite of the improvement, SOD levels were still significantly lower than the controls.

GPx was significantly lower in the malnourished infants, as well as in each sub-

Table 3 **Comparison of superoxide dismutase (U/L) levels for all groups before and after nutritional rehabilitation**

Group status	Controls	Infants with protein energy malnutrition			
		All cases	Marasmus	Kwashiorkor	MK
Before rehabilitation	368 ± 16.79	43 ± 7.67	33.5 ± 9.17	47.5 ± 16.47	50 ± 9.57
After rehabilitation		193 ± 13.45	185 ± 52.53	190 ± 22.6	207 ± 29.07
P-value		$P_1 < 0.001$	$P_1 < 0.001$	$P_1 < 0.001$	$P_1 < 0.001$
		$P_2 < 0.01$	$P_2 < 0.01$	$P_2 < 0.01$	$P_2 < 0.01$
		$P_3 < 0.001$	$P_3 < 0.001$	$P_3 < 0.001$	$P_3 < 0.05$

Results given for levels of superoxide dismutase (U/L) before and after nutritional rehabilitation are the mean level ± standard error of the mean.
MK = marasmic kwashiorkor.
P_1 *= the studied group versus controls before nutritional rehabilitation.*
P_2 *= the studied group versus controls after nutritional rehabilitation.*
P_3 *= the studied group before versus the studied group after nutritional rehabilitation.*
$P < 0.05$ *was considered significant.*

Table 4 **Comparison of glutathione peroxidase (U/L) levels for all groups before and after nutritional rehabilitation**

Group status	Controls	Infants with protein energy malnutrition			
		All cases	Marasmus	Kwashiorkor	MK
Before rehabilitation	543 ± 35.91	294 ± 25.04	317 ± 50.63	267 ± 37.57	306 ± 44.68
After rehabilitation		504 ± 28.88	513 + 38.60	507 ± 50.86	489 ± 66.66
P-value		$P_1 < 0.001$	$P_1 < 0.05$	$P_1 < 0.001$	$P_1 < 0.001$
		$P_2 > 0.05$	$P_2 > 0.05$	$P_2 > 0.05$	$P_2 > 0.05$
		$P_3 < 0.001$	$P_3 < 0.001$	$P_3 < 0.001$	$P_3 < 0.05$

Results given for levels of glutathione peroxidase (U/L) before and after nutritional rehabilitation are the mean level ± standard error of the mean.
MK = marasmic kwashiorkor.
P_1 *= the studied group compared to controls before nutritional rehabilitation.*
P_2 *= the studied group compared to controls after nutritional rehabilitation.*
P_3 *= the studied group before nutritional rehabilitation compared to the studied group after nutritional rehabilitation.*
$P < 0.05$ *was considered significant.*

group when compared to the controls, with no difference between the subgroups. Following nutrition rehabilitation, GPx was significantly increased in all patients and showed no significant difference compared to the controls. These results agree with those reported by Ashour et al. [*12*], in which GPx deficiency in PEM was explained by either consumption in peroxidation reactions due to oxidative stress, or as being due to a deficiency of selenium in the cases studied.

Table 5 **Correlation between the rate of change of SOD and GPx levels, and the rate of change of the Z-score, haemoglobin %, albumin, total leukocyte count and alanine aminotransferase levels in the malnourished infants**

Antioxidant variable	Superoxide dismutase		Glutathione peroxidase	
	r	*P*	*r*	*P*
Z-score	0.715	< 0.001	0.718	< 0.001
Haemoglobin %	0.848	< 0.001	0.925	< 0.001
Albumin	0.593	< 0.001	0.478	< 0.001
Total leukocyte count	–0.626	< 0.001	–0.549	< 0.001
Alanine aminotransferase	–0.497	< 0.001	–0.480	< 0.001

r = correlation coefficient.
P < 0.05 was considered significant.

Table 6 **Comparison of anthropometric measurements before and after nutritional rehabilitation in malnourished infants**

Variable	Before nutritional rehabilitation	After nutritional rehabilitation	*P*-value
Weight (kg)	4.96 ± 0.26	5.87 ± 0.29	< 0.001
Length (cm)	63.8 ± 1.32	64.2 ± 1.30	< 0.05
Z-score	–2.2 ± 0.29	–0.62 ± 0.31	< 0.001
Mid-arm circumference (cm)	9.42 ± 0.29	10.0 ± 0.33	< 0.05
Skin-fold thickness (mm)	4.45 ± 0.59	4.70 ± 0.65	< 0.05

Values are expressed as the mean ± standard error of the mean.
P < 0.05 was considered significant.

The rate of change of SOD and GPx showed significant positive correlation with the rate of change of each of the Z-score, the albumin level and Hb %, and a significant negative correlation with both total leukocyte count and ALT.

These correlation tests clearly demonstrate the role of antioxidants in the pathogenesis of the various features of PEM. The rate of improvement of the SOD and GPx correlated with the improvement in anthropometric measures represented here by the Z-score. With regard to albumin, Huang and Fwu [*13*] observed that the oxidative stress in PEM resulted in an increase in lipid peroxidation that might in turn lead to a decrease in serum albumin levels. During nutrition rehabilitation, albumin levels significantly increased back to normal and its rate of change correlated positively with that of the enzymatic antioxidants.

An imbalance between proxidant and antioxidant defence has also been implicated in PEM anaemia [*14*]. The finding of

Table 7 **Comparison of laboratory parameters before and after nutritional rehabilitation in malnourished infants**

Variable	Before nutritional rehabilitation	After nutritional rehabilitation	*P*-value
Total leukocyte count	10.55 ±0.70	9.83 ± 0.77	< 0.05
Haemoglobin % (g/dL)	7.76 ± 0.25	9.91 ± 0.12	< 0.001
Total proteins (g/dL)	5.34 + 0.21	6.78 ± 0.12	< 0.001
Albumin (g/dL)	2.30 ± 0.49	3.48 ± 0.08	< 0.001
Alanine aminotransferase (IU/L)	40.8 ± 4.47	38.7 ± 7.14	> 0.05
Creatinine (mg/dL)	0.45 ± 0.02	0.47 ± 0.02	> 0.05

[a]Values are expressed as the mean ± standard error of the mean.
$P < 0.05$ was considered significant.

anaemia in the malnourished infants in our study is similar to that reported by Ashour et al. [*12*]. The Hb level improved significantly on nutrition rehabilitation and its rate of change correlated positively with that of the enzymatic antioxidants further confirming the intimate relationship between PEM anaemia and oxidant-antioxidant imbalance.

Infection and inflammatory toxins are potent stimulators of free radical formation [*5*]. Total leukocyte count counts were significantly increased in the malnourished infants denoting infection and sCRP was positive in all the patients on admission. Osman et al. [*15*] has previously confirmed the close relationship between nutritional status and incidence and severity of infections, whether acute respiratory infections or gastroenteritis. During nutrition rehabilitation, total leukocyte count significantly decreased due to infection control, thus its rate of change correlated negatively with that of enzymatic antioxidants.

Fatty liver, a hallmark of hepatic free radical damage [*16*], develops on a low protein diet and is directly related to impaired hepatic function [*17*]. This explains the high ALT levels in the patients in our study which decreased during nutritional rehabilitation. Thus, the rate of change of ALT correlated negatively with the rate of change of SOD and GPx, further incriminating the oxidant-antioxidant system derangements in the pathogenesis of PEM-related hepatic pathology.

From the course of this study, it is evident that there is a significant correlation between enzymatic antioxidants and the clinical and laboratory parameters that reflect the pathological features of PEM. We conclude that enzymatic antioxidants could be used as a prognostic tool in PEM, as well as a marker for severity. The compromised enzymatic antioxidant status in PEM can be reversed successfully by proper nutritional rehabilitation. Therefore, there should be special emphasis on supplying antioxidant agents during any PEM rehabilitation programme, such as vitamins (A, E and C) and trace elements (copper, zinc and selenium). Consideration should also be given to supplying therapeutic enzymatic antioxidants in order to reduce free radical generation and enhance antioxidant status. These measures may help to reduce the mortality and morbidity associated with PEM.

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